PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Gianfranco MERIZZI
Appln. No.: 10/589,469
Confirmation No.: 7234
Filed: August 14, 2006

Docket No.: 52290

Group Art Unit: 1617
Examiner: Zarek, Paul E.

For: USE OF N-PIPERIDINE DERIVATIVES FOR THE TREATMENT OF NEURODEGENERATIVE PATHOLOGIES

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. BOX 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Moreno Paolini, hereby declare as follows.

I am full professor of Pharmacology and Toxicology at the University of Bologna, my research focuses on xenobiotic metabolism – at the genotype and phenotype level – and on the mechanism of oxidative protection of biological systems; a list of my publications (184 full papers, 65 short communications, 174 abstracts, 44 others in national journals) is available from the website www.unibo.it/docenti/moreno.paolini.

I am acting as scientific advisor for Medestea Internazionale S.r.l., co-applicant of WO2005/084677 from which the above-identified US application derives and I am familiar with the content of said US application.

I am also familiar with the rejection of the presently pending claims of the above-identified US application under 35 U.S.C. § 103(a) as being unpatentable over Paolini and Pedulli, in view of Ito et al., Floyd et al. and Atlas et al..

I am also familiar with the rejection under 35 U.S.C. § 112, first paragraph, based on lack of an enabling disclosure.

I am co-inventor of the US patent, 5,981,548, cited as the primary reference in the rejection under 35 U.S.C. § 103(a) and I am therefore fully familiar with its disclosure.

In US 5,981,548, Dr. Pedulli and the Undersigned have disclosed the oxygen radical scavenger activity of the cyclic hydroxylamines of Formula (1) of the present invention; however, the description of US 5,981,548 does not provide any test or evidence which may suggest the utility of said compounds for the therapeutical treatment of neurodegenerative diseases. In fact, the disclosure of US 5,981,548 only refers to said compounds as pharmacological agents to be selected *"to capture the oxygen-free radicals which are associated to a number of different human pathologies, such as phlogistic processes, alcoholic hepatopathy, liver transplants, metabolic sicknesses, alterations in lipoproteins, lung pathologies, hematologic disorders, glomerule pathology, spermatozoa pathology, coronary atherosclerosis, hyperbaric damages affecting the central nervous system, radiation damages, DNA damages by genotoxines, oxidative polymorphisms, inductive status, etc."* (column 6, lines 32-41).

The quoted passage does not disclose the utility of the compounds for the therapeutical treatment of said pathologies and this is further confirmed by the description (column 6, lines 43-46) which discloses *"the use of the above-mentioned compound for preparing a pharmaceutical composition for treating symptoms due to excess of production of superoxide radicals"* (emphasis added).

The description of US '548 provides no disclosure or suggestion to a person of ordinary skill in the art for the use of said compounds for the therapeutical treatments of neurodegenerative diseases or the treatment or inhibition of the symptoms of Parkinson's disease or ischemia/reperfusion injury.

On the other hand, it is my opinion that the disclosure of the specification of the above-captioned patent application provides evidence which would be considered by the person of ordinary skill in the art as enabling for the treatment of a neurodegenerative disease and for the treatment or inhibition of symptoms of Parkinson's disease and the symptoms of ischemia/reperfusion injury.

On the basis of said disclosure, further additional tests have been carried out, under my supervision as scientific advisor, which have confirmed the utility of the compound bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)decandioate, therein identified as IACVITA or IAC, for the therapeutical treatment of neurodegenerative diseases and other diseases mentioned by the claims of the above-identified application.

With specific reference to neurodegenerative diseases, I enclose herewith:

- Exhibit 1. A presentation reporting a summary of preclinical data on Alzheimer disease, carried out by the company Cerebricon on behalf of Medestea: The tests relate to the effect of IAC-VITA on neurite outgrowth in vitro, and to the effect of IACVITA treatment on single transgenic mouse model of Alzheimer disease. The data confirm the excellent activity of IACVITA in contrasting neurobehavioral deficits linked with the progression of Alzheimer disease.

- Exhibit 2. A presentation providing a summary of preclinical data on stroke and brain ischemia including:
  - a study on the protective effect of IAC on bilateral common carotidal artery occlusion (BCCO) post-ischemic brain damage in Mongolian gerbils, carried out by the Faculty of Pharmacy of the University of Catanzaro on behalf of Medestea;
  - a study on the effect of i.p.IAC treatment on infarct volume and sensory-motor behaviour in tMCAO rats, carried out by the company Cerebricon on behalf of Medestea;
  - a study on the effect of i.v.IAC treatment on infarct volume and sensory-motor behaviour in tMCAO rats, carried out by Cerebricon;
  - a study on the effect of i.v.IAC treatment on infarct volume in tMCAO mice, carried out by Cerebricon;
  - a study on the effect of the late i.v.IAC treatment on infarct volume in tMCAO rats carried out by Cerebricon; and
  - a study on stroke prevention with IAC in Dahl salt-sensitive rats.

With specific reference to the pathologies claimed in presently pending claim 6 of the above-identified application, I enclose herewith:

- Exhibit 3. a presentation providing the results of a study on the *in vivo* and *in vitro* IAC activity on diabetes models, carried out by the Department of Endocrinology and Metabolism of the University of Pisa;

- Exhibit 4. a presentation providing the results of a study on the protective effect of IAC on balloon injury related neointima formation, carried out by the Faculty of Pharmacy of the University of Catanzaro;

- Exhibit 5. a presentation providing the results of a study on the protective effect of IAC on cardiac ischemia: ischemia reperfusion in the isolated perfused Langerdorff heart, carried out by the Faculty of Pharmacy of the University of Catanzaro;

- Exhibit 6. a presentation providing the results of a study on the effects of IAC on myocardial ischemia and reperfusion on rats, carried out by Pharma Hungary;

- Exhibit 7. a presentation providing the results of a study on the anti-hypertensive activity of IAC, carried out by the Faculty of Pharmacy of the University of Catanzaro;

- Exhibit 8. a presentation providing the result of a study on the effects of IAC on *in vivo* oral mucositis induced by acute radiation in Hamsters, carried out by Biomodels LLC and Affiliates;

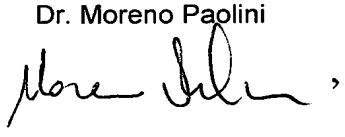
- Exhibit 9. a presentation relating to the effects of IAC on an *in vivo* sepsis induction in rat models, carried out by Eurofins-Product Safety Laboratories.

The data presented in Exhibits 1-9 and the present specification demonstrate that one skilled in the art would have a reasonable expectation of success in treating a neurodegenerative disease and the treatment or inhibition of the symptoms of Parkinson's disease or ischemia/reperfusion injury, as well as in the treatment of the specific pathologies listed in claim 6.

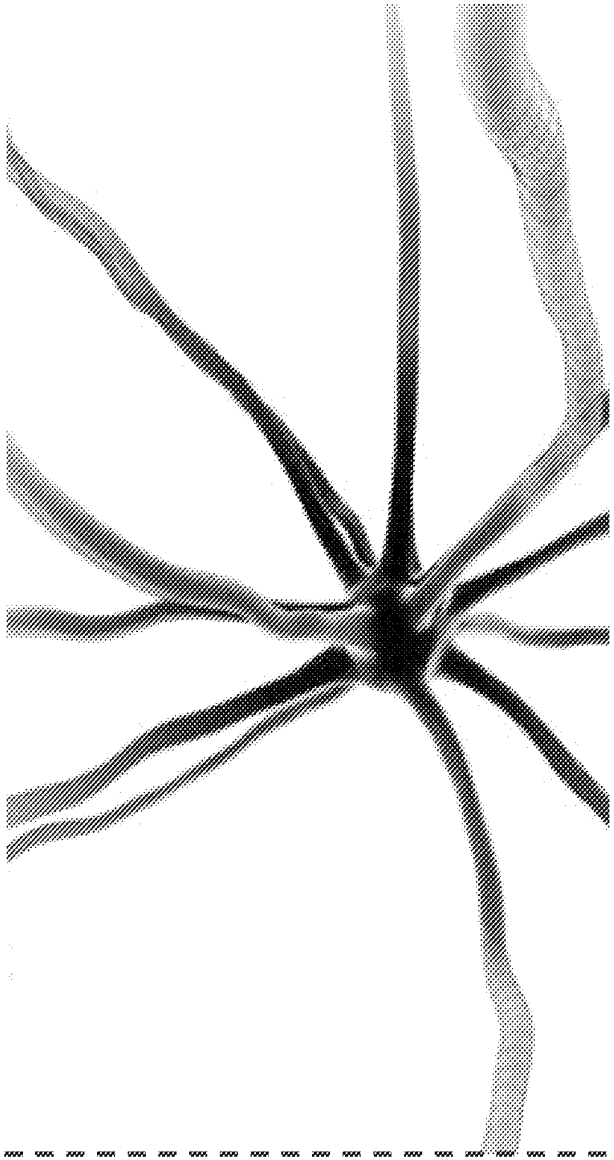
I declare further that all statements made herein on my own knowledge are true and that all statements made, on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, both, under section 1001 Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

Date: February 25, 2010

Dr. Moreno Paolini



Encl.:
- presentations of studies



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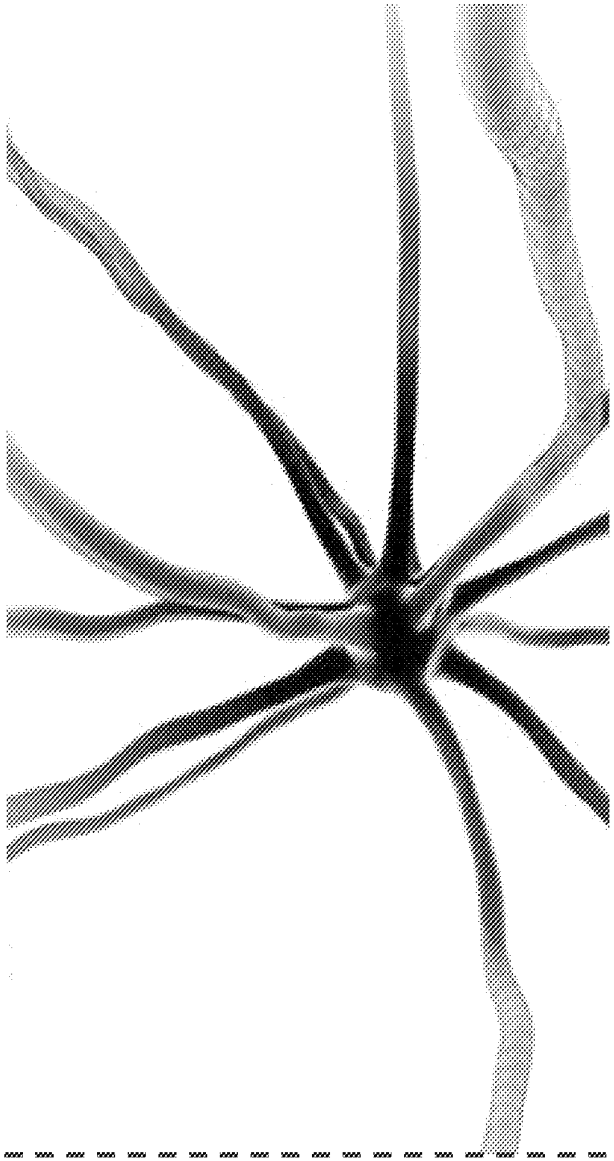
IACVITA

SUMMARY OF PRECLINICAL DATA ON ALZHEIMER DISEASE

EXHIBIT 1



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Cerebricon

A leader in the non-clinical screening of drug candidates against CNS disease targets.

*Effect of **IACVITA** on Neurite Outgrowth in vitro*

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Study outline

- E18 Wistar rat embryos mixed cortical cells
- 5 doses: 0.01, 0.1, 1, 10, 100 μM IAC
- NGF (50 ng/ml) as positive control
- Time window: 4 hours after plating
- Evaluation of neurite outgrowth

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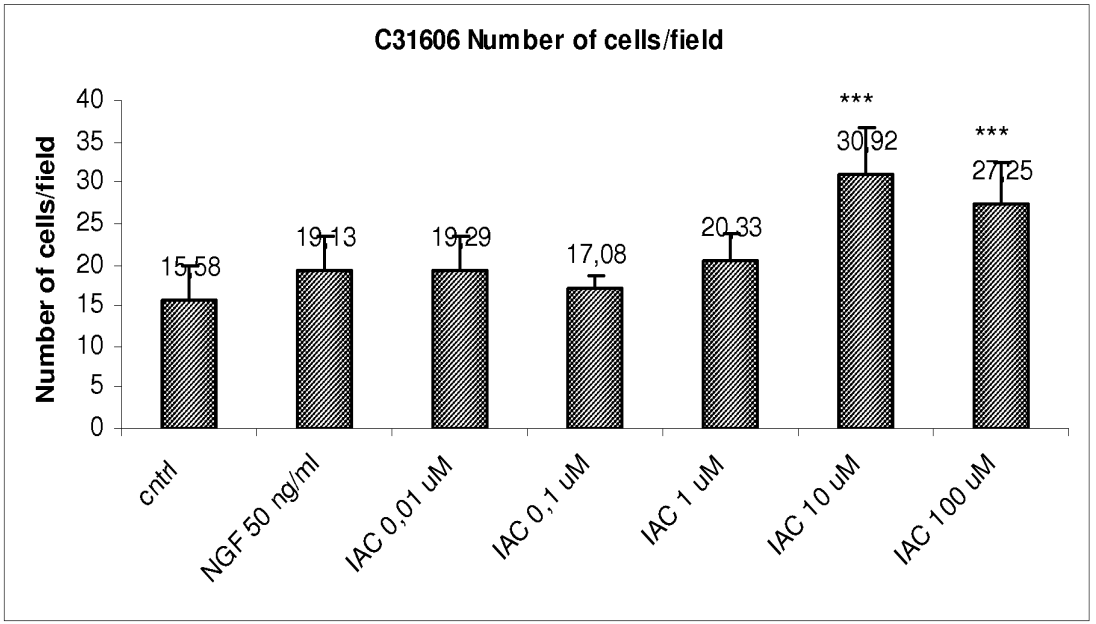
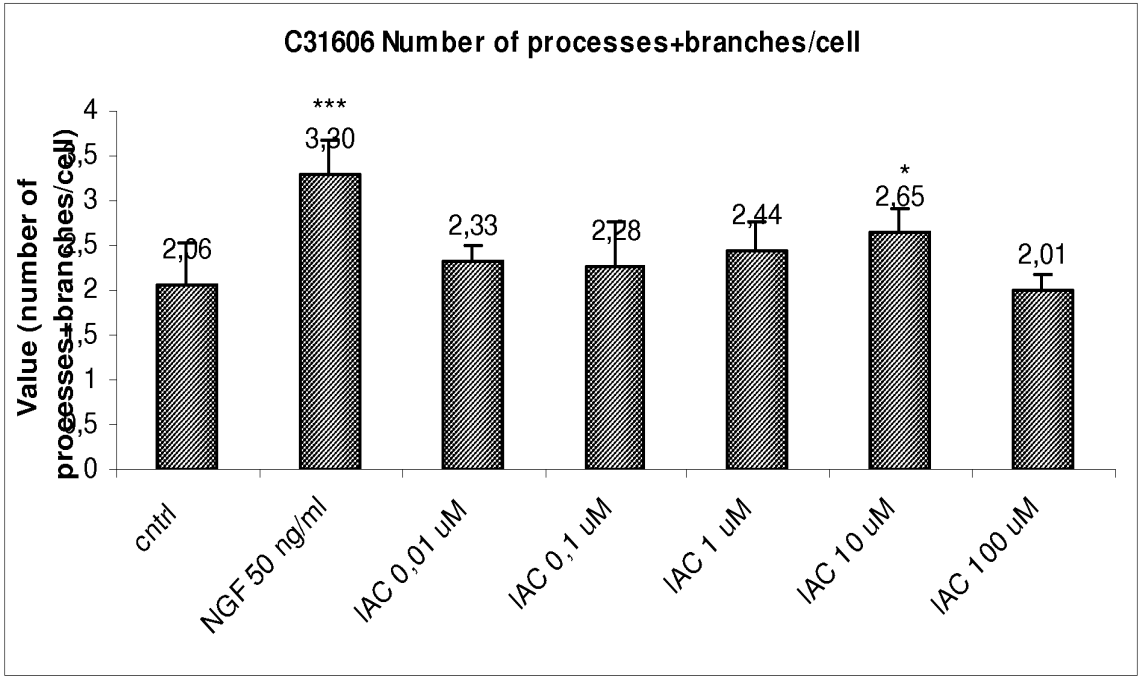
Results



***p<0,001 (1-way ANOVA)



*p<0.05 and ***p<0,001
(1-way ANOVA)

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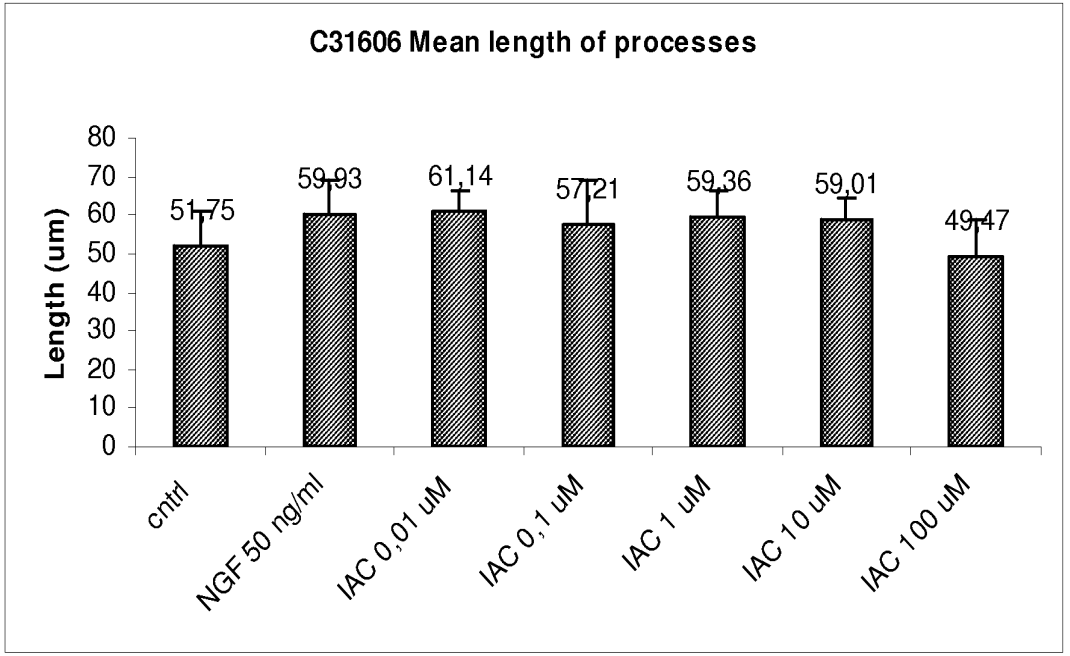
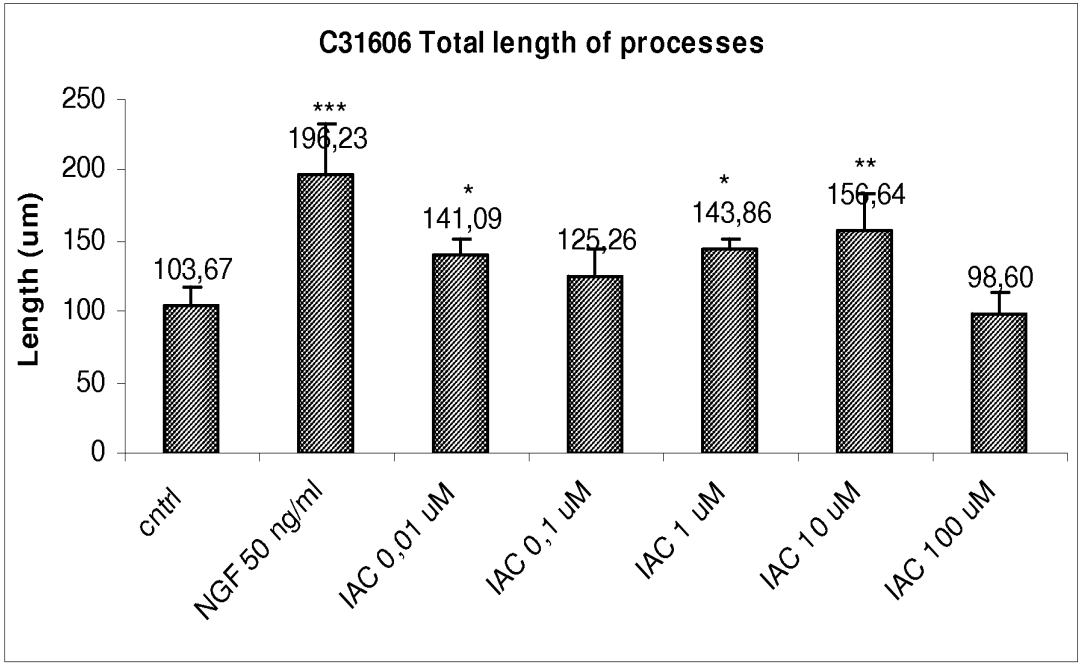
Results





*p<0.05, **p<0.01 and ***p<0,001 (1-way ANOVA)

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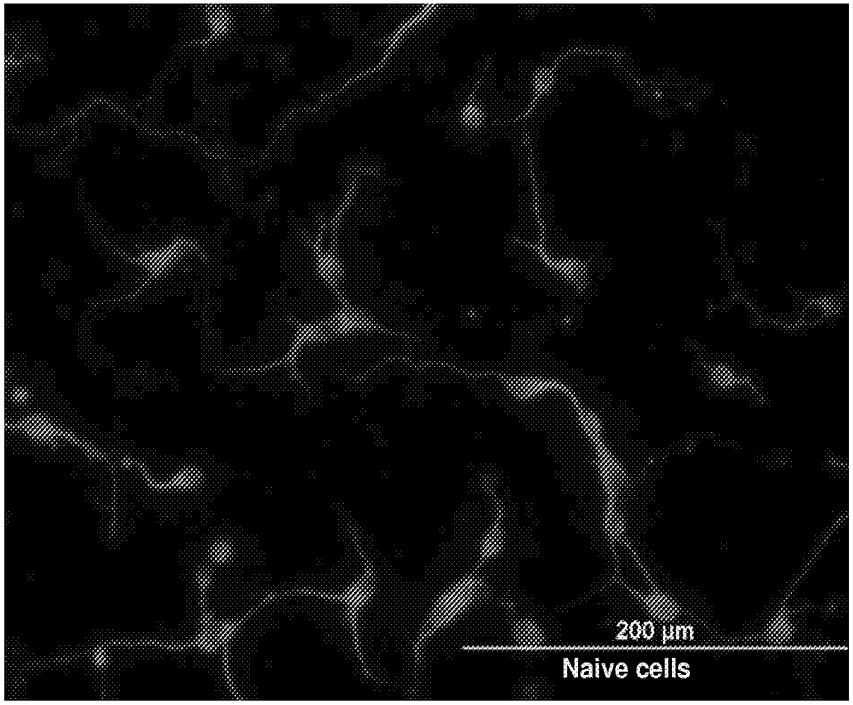
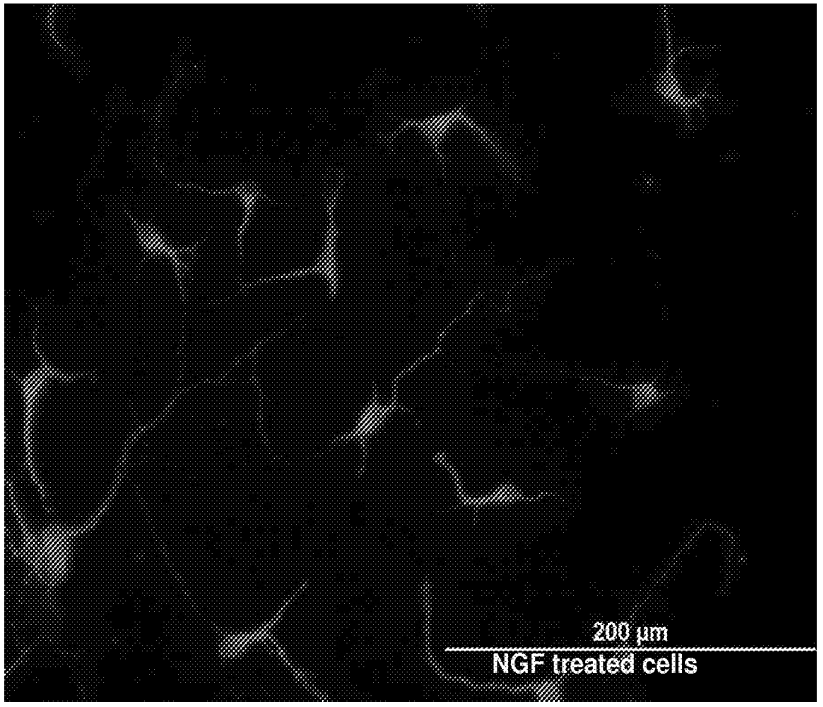
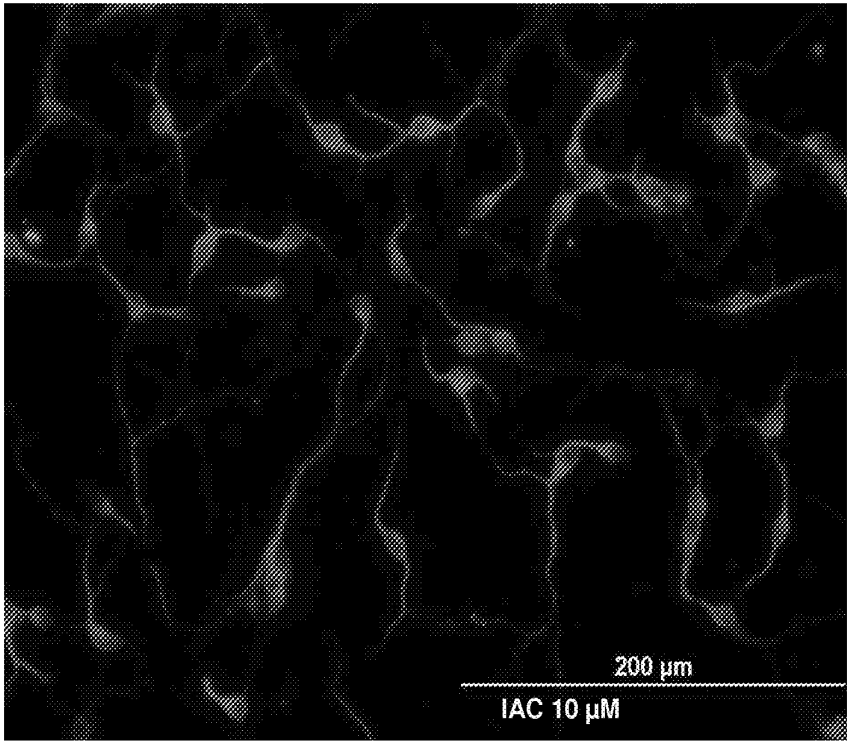
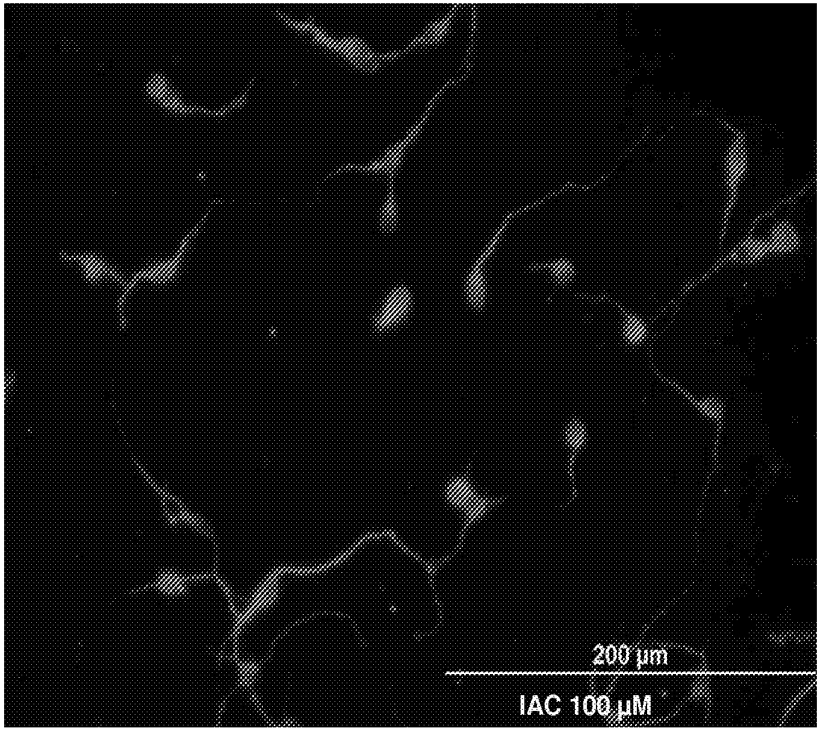

200 µm
Naive cells


200 µm
NGF treated cells


200 µm
IAC 10 µM


200 µm
IAC 100 µM

Conclusions

- IAC at 10 and 100 µM significantly increased the number of cells per field, meaning that IAC at these concentrations decreased the early-phase cell death in this system;

- IAC at 10 µM was also able to significantly increase the number of neurites and neurite branches per cell;

- These results suggest that IAC is able to prevent the early-phase cell death occurring in mixed cortical neurons, increase the number of neurites and neurite branches in cells as well as increase the total neurite length *in vitro*.

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2007 – San Diego, California, Nov. 3-7

SOCIETY FOR NEUROSCIENCE

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IAC promotes neurite outgrowth in cortical neurons and influences production of inflammatory markers in glia *in vitro.*

***N. E. VARTIAINEN[1], A. SOLETI[2], M. PAOLINI[3], F. RICCARDINO[2], A. ARMANDO[2], J. YRJÄNHEIKKI[1], R.I. GRUNDY[1];**

[1]Cerebricon Ltd, Kuopio, Finland; [2]Medestea Res., Torino, Italy; [3]Univ.of Bologna, Bologna, Italy

Background

IAC is a free radical scavenger which interacts with most (if not all) carbon-, nitrogen- and oxygen-centered radicals of biological interest – including peroxyl, superoxide, and peroxynitrite radicals. Furthermore, thanks to its peculiar physical-chemical properties, IAC is easily distributed in both extracellular and intracellular compartments. We have previously shown that IAC is neuroprotective and restores function following cerebral ischemia in the rat. The present study seeks to further investigate the mechanism of action behind this protection and functional recovery by observing the effects of IAC on neurite outgrowth and inflammation in neuronal cells.

Materials & Methods

Neurite Outgrowth Assay

- The cortical mixed cultures were prepared from E18 Wistar rat embryos After the cells had attached to the well, 250 µl medium was added to the wells. Four hours after plating the medium was changed to fresh medium containing IAC at 0,01-100 µM. Mouse nerve growth factor (NGF, 50 ng/ml) was used as a positive control. After 2 days *in vitro*, the cells were formaldehyde-fixed and processed for immunocytochemistry.
- The cultures were fixed with 4% formaldehyde in 0.01 M PBS for 30 min and washed once with PBS. Rabbit anti-MAP-2 (dilution 1:1000, Chemicon, in blocking buffer) was used as a primary antibody. The cells were incubated with the primary antibody for 48 h at +4°C, washed with PBS, and incubated with secondary antibody goat anti-rabbit IgG conjugated to Alexa Fluor568 (A11036, Molecular Probes) for 2 h at RT. The number of cells per field (4 fields per well) was counted, and the neurite outgrowth was quantified using Image Pro Plus software.

LPS Assay

- The cortical glial cultures were prepared from newborn Wistar rat pups . Cells were plated on 75 cm2 cell culture flasks, in MEM supplemented with 2 mM glutamine, 0,1 µg/ml gentamicin, and 10 % heat-inactivated fetal bovine serum (FBS-HI). After 7 days, the medium was changed to fresh medium. After 14 days *in vitro*, the cells were trypsinated (0.25 % trypsin/EDTA) and re-plated on 96-well-plates. The cultures were fed once before experiment.
- IAC was pipetted on wells 30 min before adding 1 µg/ml (final concentration) LPS for a period of 24 hours.
- Nitrite determination was performed according to the Griess method.. The absorbance was measured at 540 nm.
- Determination of inflammatory mediators IL-1β and TNF-α were performed according to the manufacturer's instructions. Both ELISA kits were purchased from R&D Systems.

Results

- IAC at 10 and 100 µM significantly decreased the early-phase cell death.
- NGF (50 ng/ml), used as a positive control for neurite outgrowth, significantly increased the number of neurites and neurite branches per cell.
- IAC at 10 µM was also able to significantly increase the number of neurites and neurite branches.
- NGF and IAC at 10 µM resulted in significant increase in total neurite length per field. In rat glial cultures
- LPS-induced nitrite production was decreased by IAC at 1mM.
- IL-1β production was significantly increased with 100 µM IAC.
- TNF-α production was significantly inhibited by IAC at concentrations as low as 1 micro M.

Conclusions

This study shows that IAC is able to prevent the early-phase cell death occurring in mixed cortical neurons, increase the number of neurites and neurite branches in cells as well as increase the total neurite length in cortical neurons *in vitro*. Further, IAC is able to modulate the inflammatory reaction caused by LPS in glia. These data suggest that IAC acts via multiple mechanisms of action relevant to the processes involved in neurodegeneration

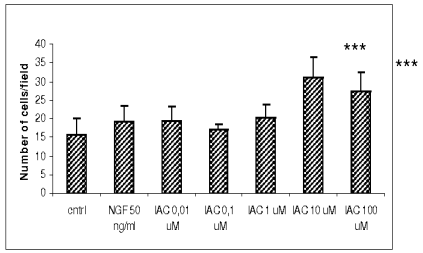


Figure 1. IAC increases survival of neuronal cells under basal conditions. Values are presented as Mean+SD. ***p<0,001 compared to control (1-way ANOVA).

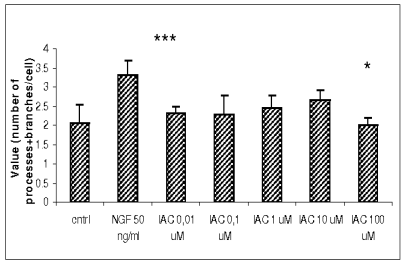


Figure 2. IAC increases the number of neurite processes and branches. Values are presented as Mean+SD. *p<0.05, ***p<0.001; respectively compared to control (1-way ANOVA).

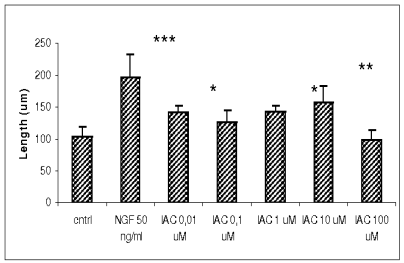
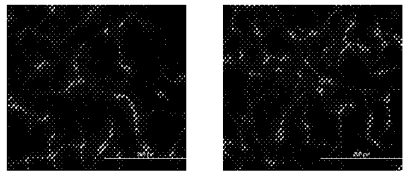


Figure 3. IAC increases the total neurite length. Values are presented as Mean+SD. *p<0.05, **p<0.01, ***p<0.001, respectively compared to control(1-way ANOVA)



A. Naive cells. Magnification 20x B. Cells treated with 10 uM IAC. Magnification 20x

Figure 4. Representative images of A. naive neuronal cells and B. neuronal cells treated with 10 uM IAC

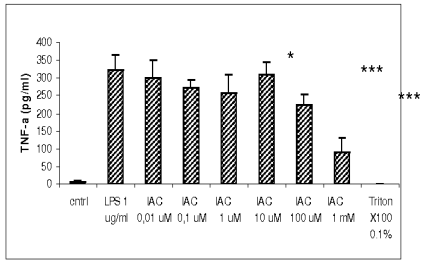


Figure 5. IAC reduces the amount of TNF-α in response to LPS stimulation in glial cells. Values are presented as Mean+SD. *p<0.05, **p<0.001, respectively (1-way ANOVA).

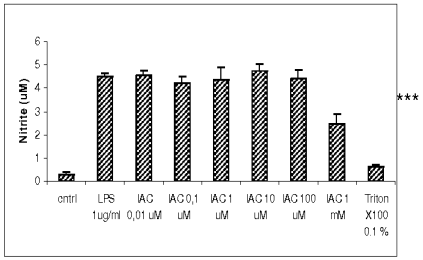


Figure 6. IAC at 1 mM decreases the amount of nitrite in response to LPS stimulation in glial cells. Values are presented as Mean+SD. ***p<0.001 (1-way ANOVA).

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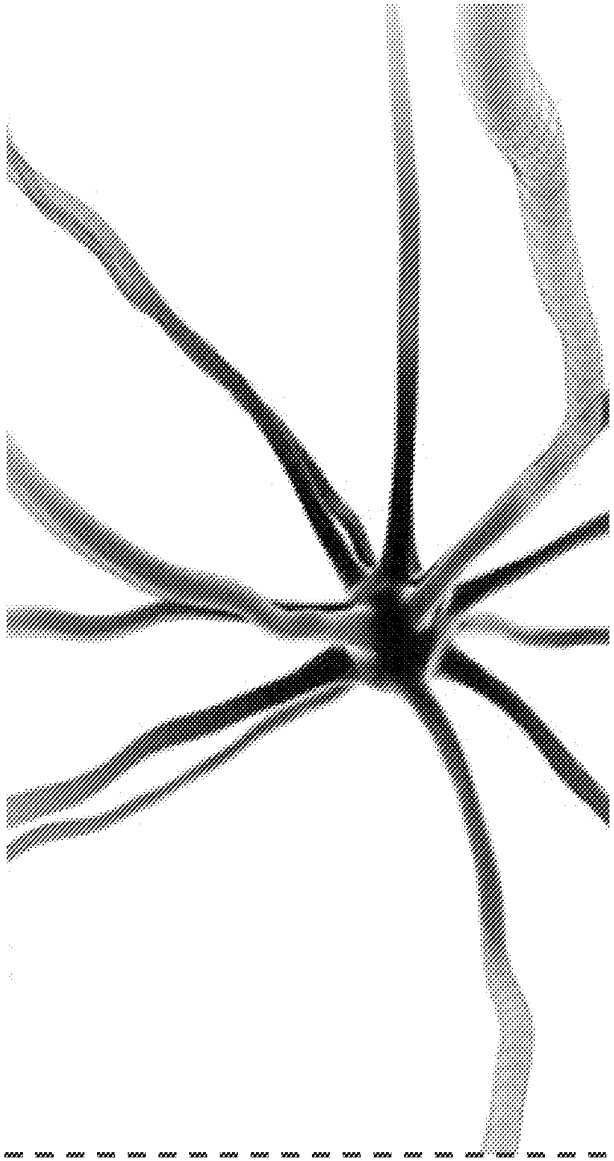

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A leader in the non-clinical screening of drug candidates against CNS disease targets.

*Effect of **IACVITA** treatment on Single Transgenic Mouse model of Alzheimer Disease*

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Study outline

- Animal model: transgenic **Tg2576 mice** (n=60) which develop amyloid plaques and progressive cognitive deficits
- 3 doses: 3, 10 and 30 mg/kg i.p. (daily injection)
- Time window: from 6 months old to 13 months old
- Evaluation of:
  - Behavioral testing: Open field test, Y-Maze, MWM, contextual fear conditioning.
  - Hystological analysis of brain to determine plaque load.

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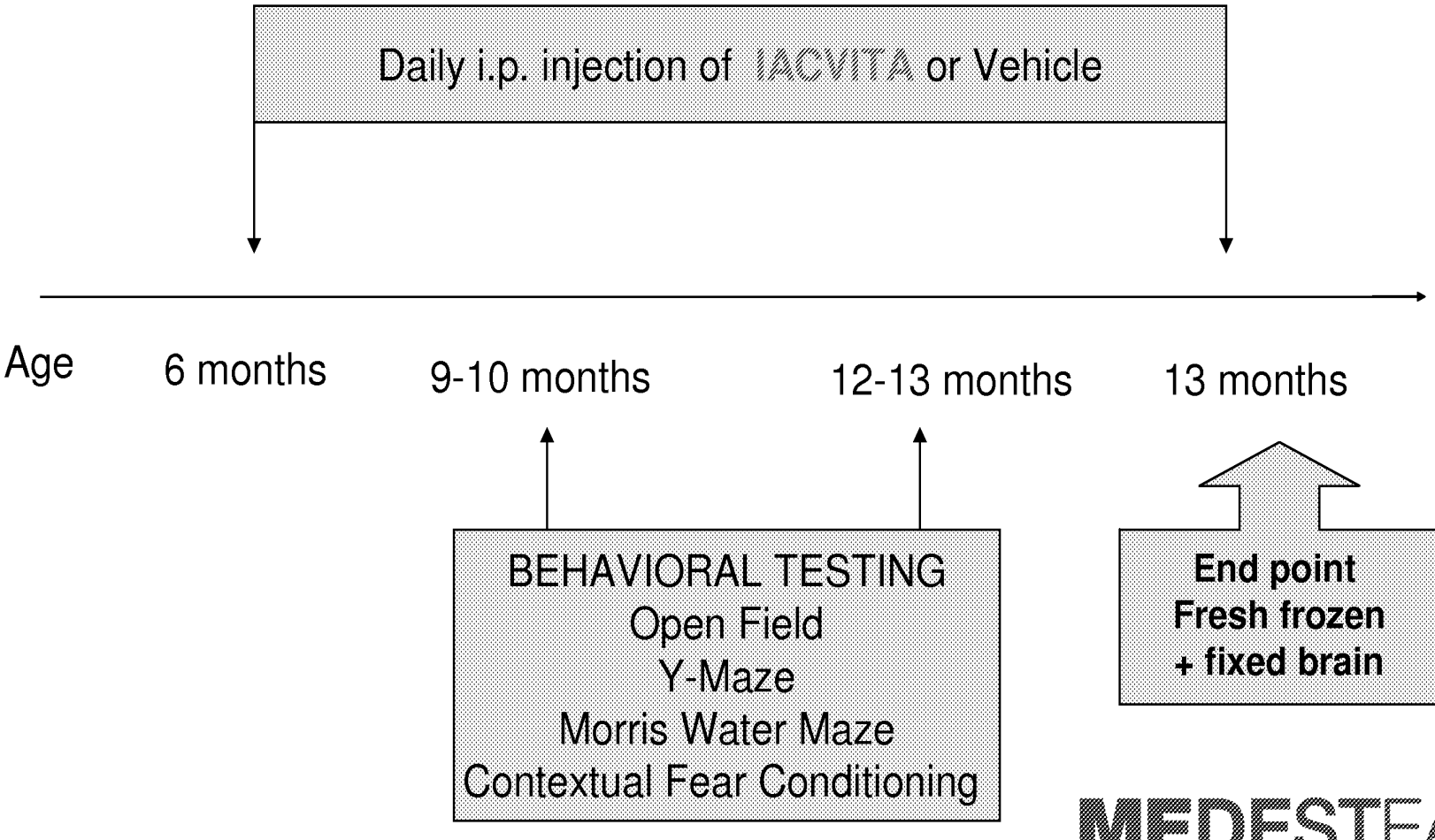
Study outline



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Results – MWM Thigmotaxys

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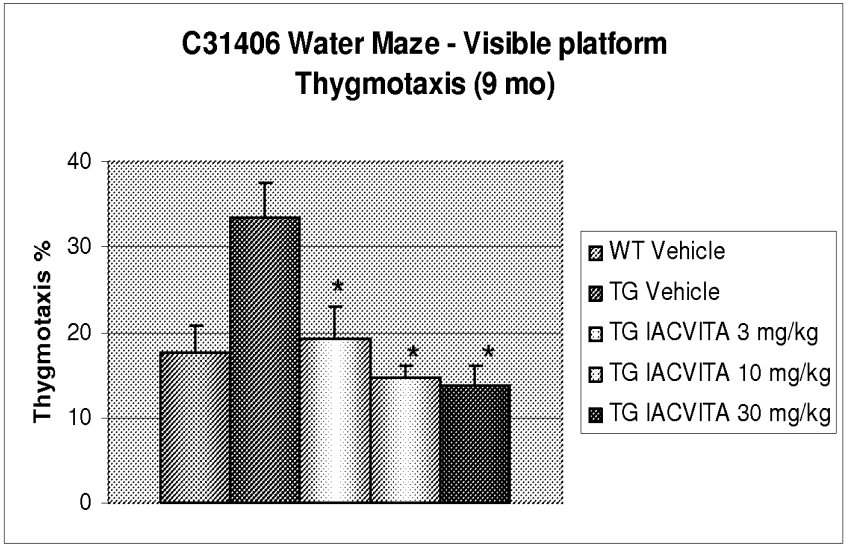

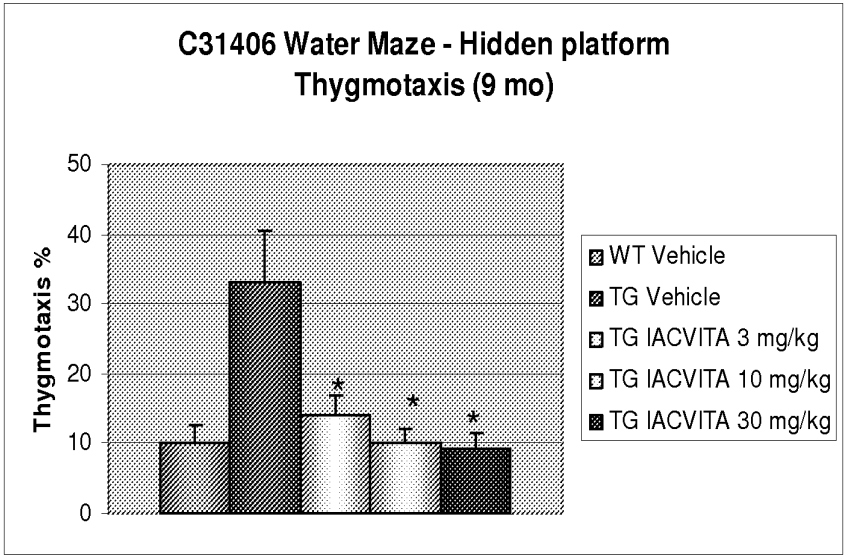

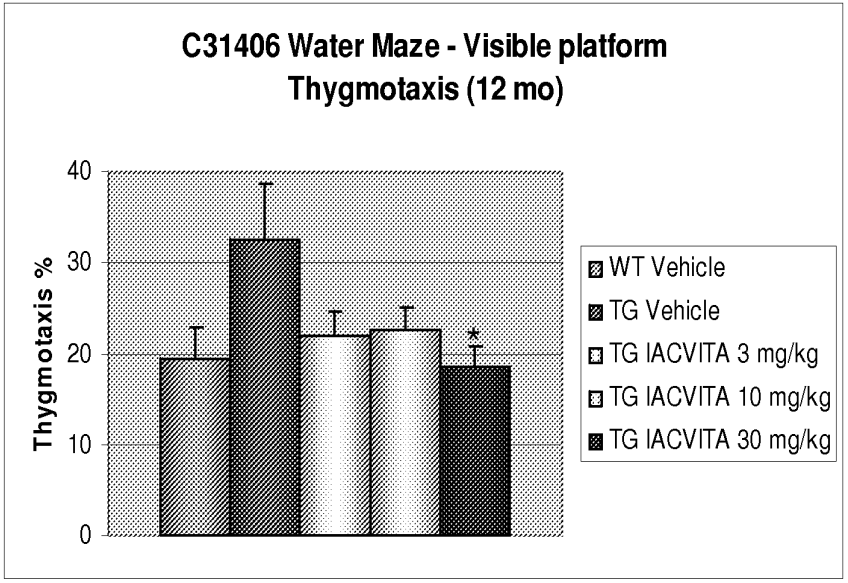

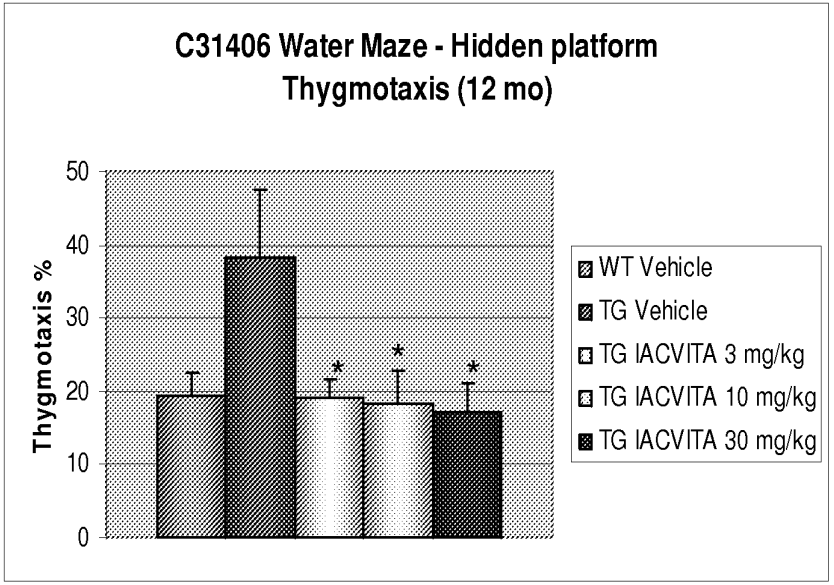

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Results – MWM Latency

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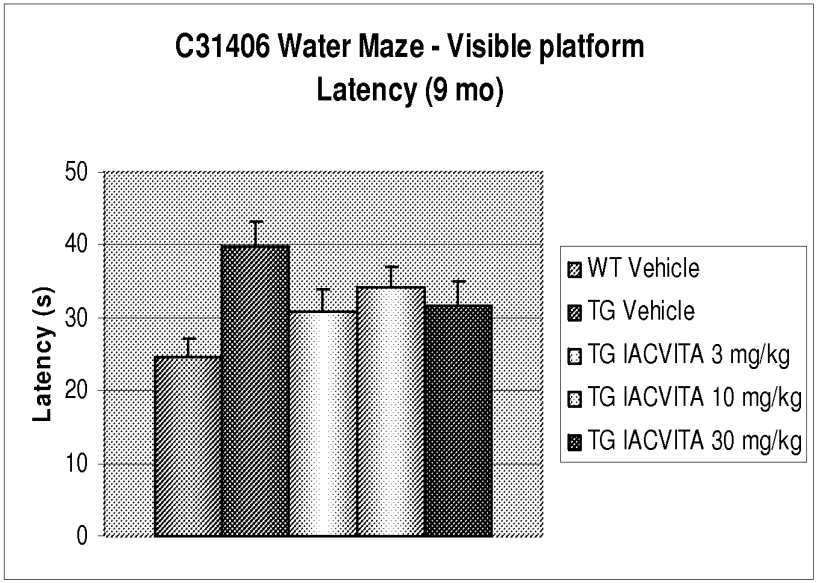

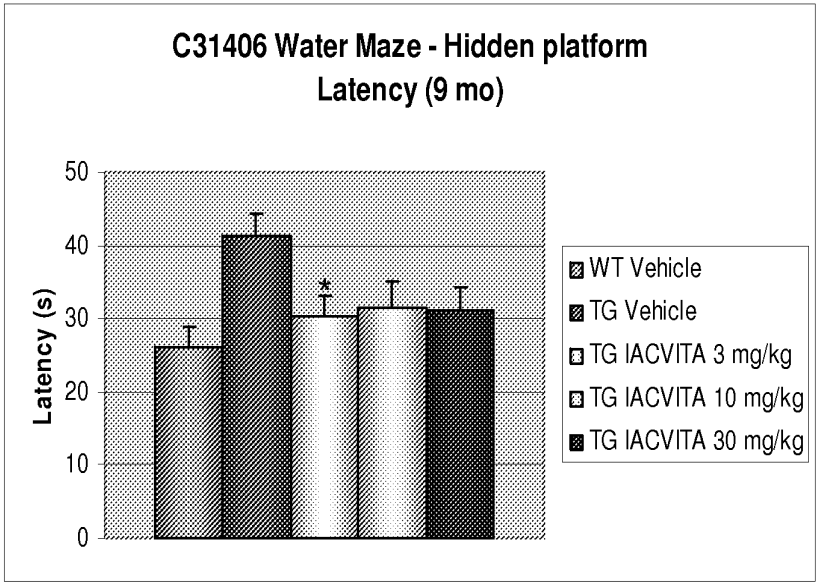

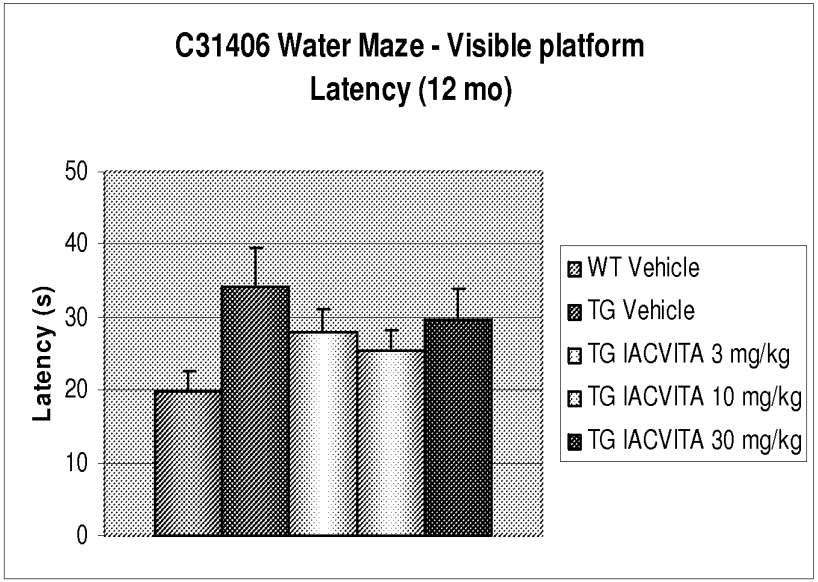

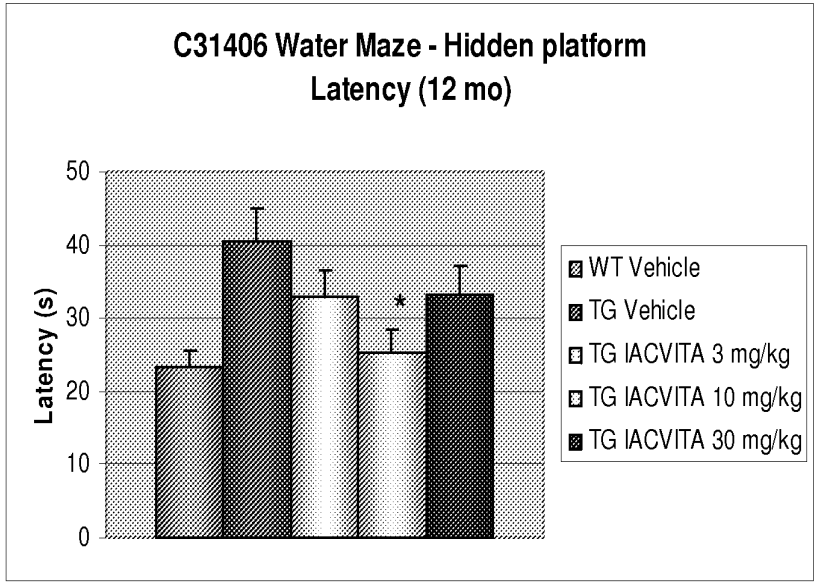

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Results – MWM Platform finding

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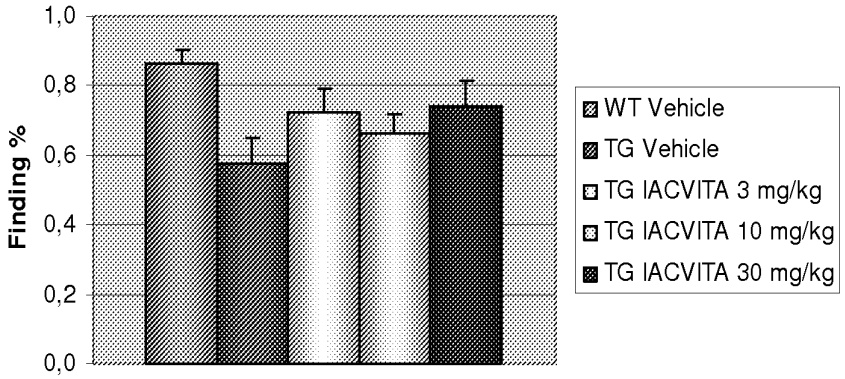

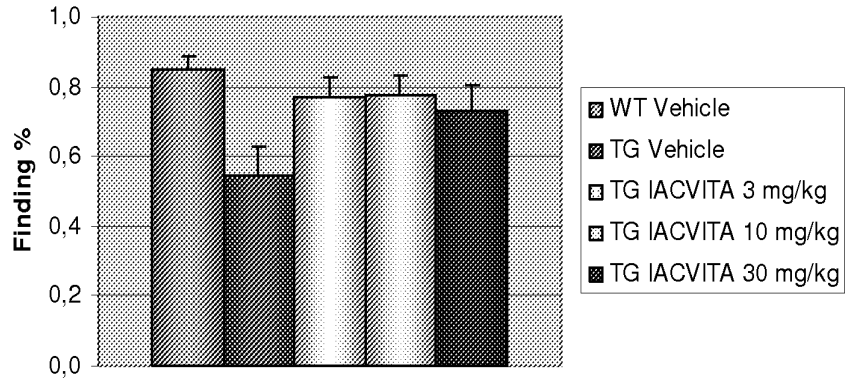

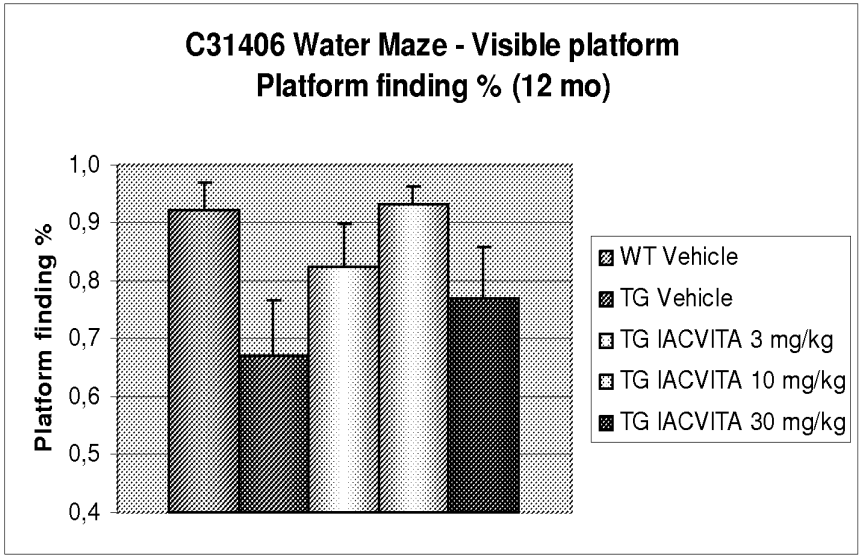

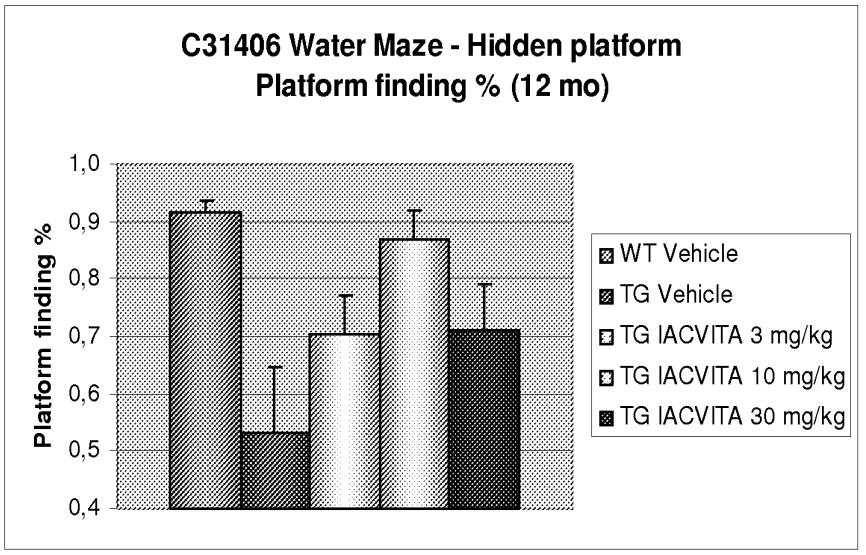

Remarkably, 10 mg/kg IAC-treated mice showed the same ability in finding the hidden platform if compared to wild-type littermates

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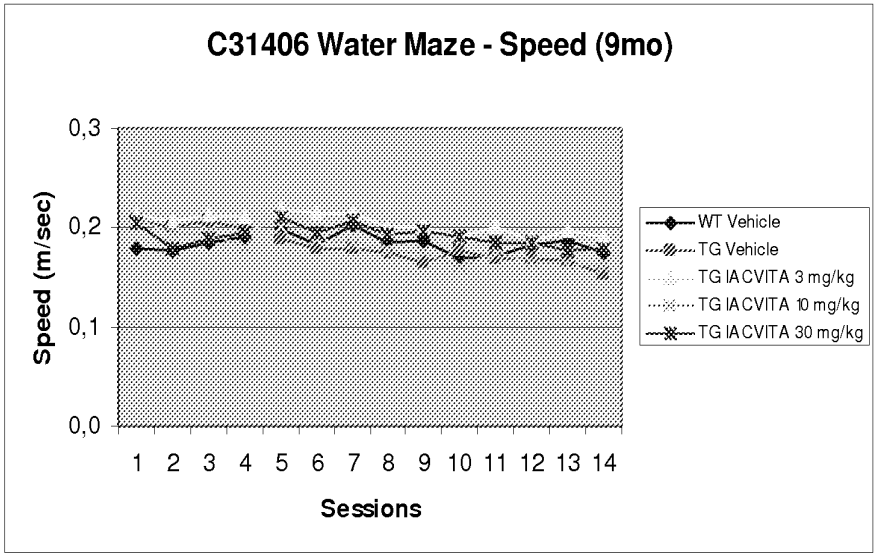
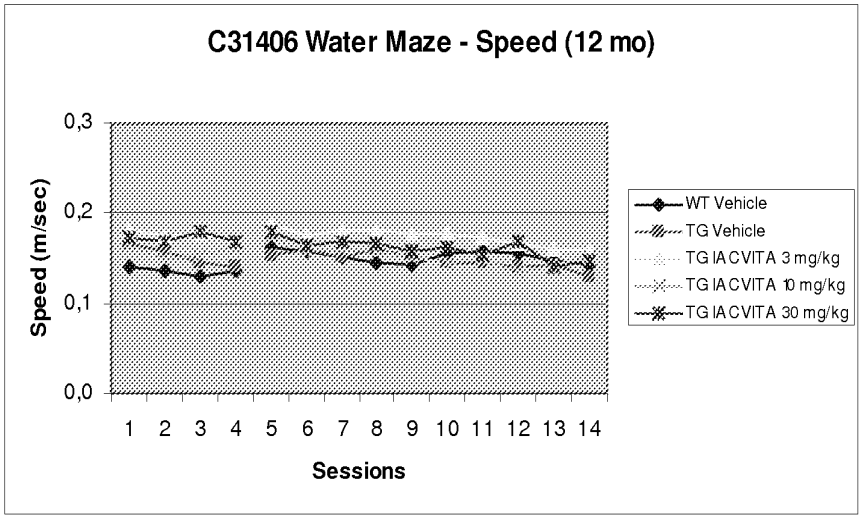
CEREBRICON

Results – Swimming Speed





There were no significant differences in swimming speed between IAC groups, TG mice and WT littermates.

This information is quite important as it is suggestive of the fact that **the treatment didn't impare the physical performances of mice.**

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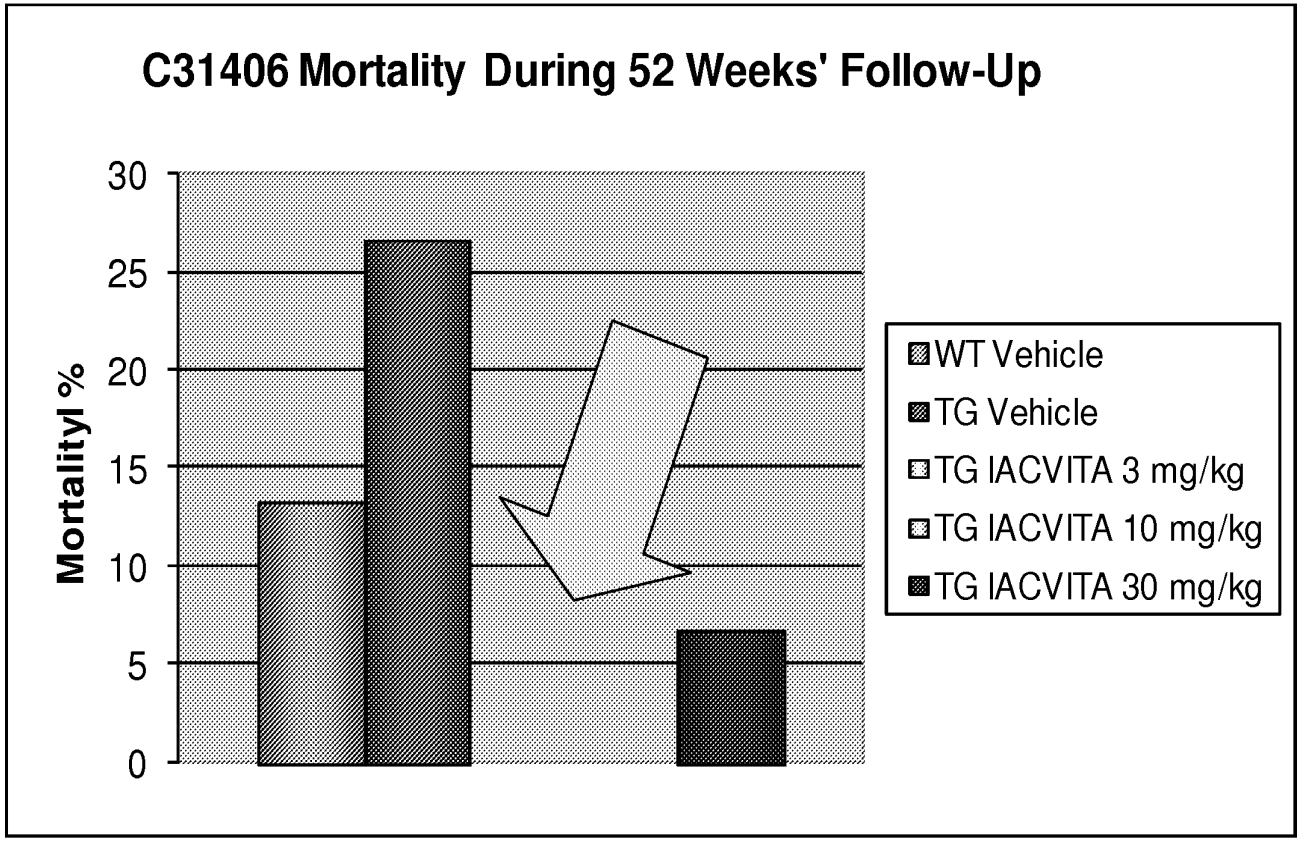
Results – Mortality



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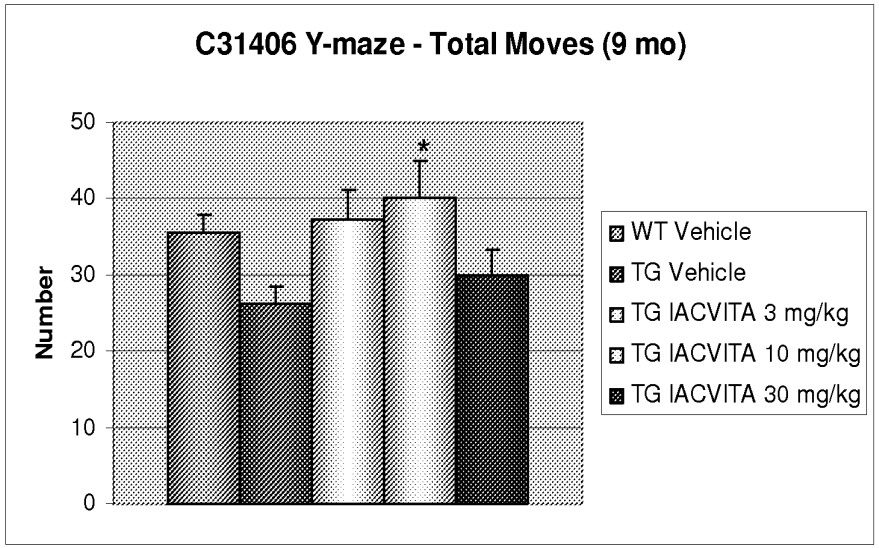
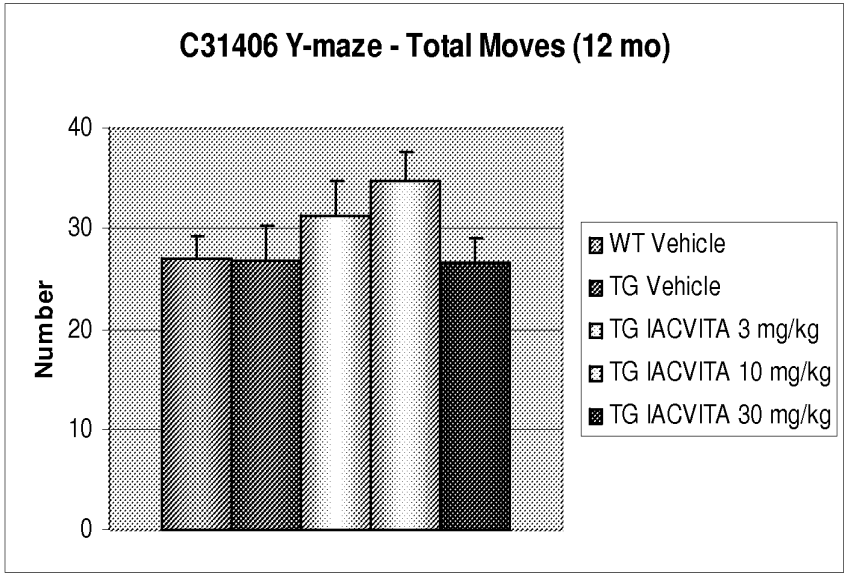
Results – Y-maze



3 mg/kg and 10 mg/kg IACVITA treatments were also effective in restoring some neurobehavioural parameters (such as total moves) in Y-maze test.



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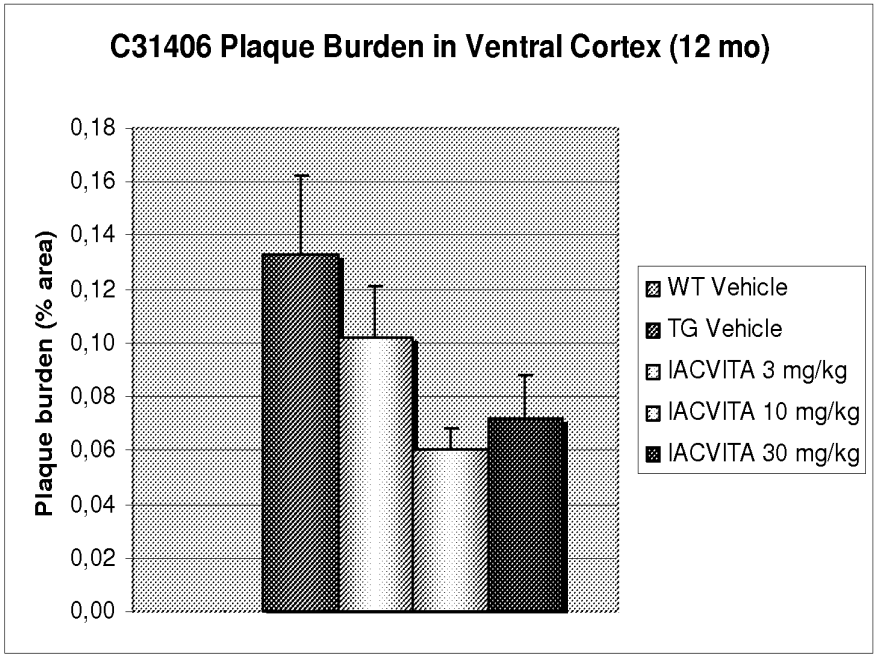
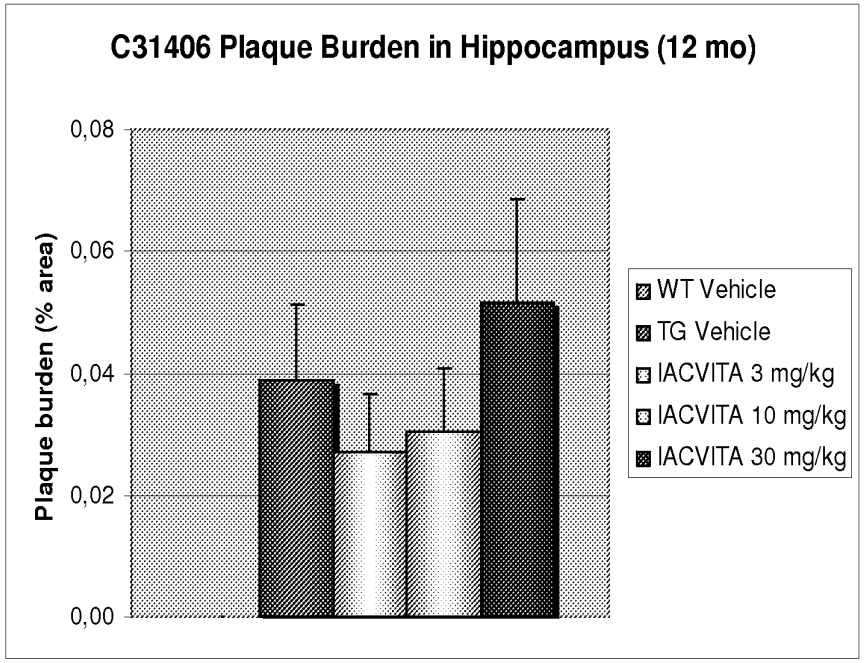
Results – Plaque load





There was a significant difference in plaque load between IACVITA groups and TG mice, particularly in ventral cortex area.

This information is rather important as it is suggestive of the fact that the treatment (7 months, daily i.p.) was able to reduce the β-amyloid aggregation.

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Conclusions

- The data gathered so far show an **excellent activity** of IACVITA in counteracting neurobehavioral deficits linked with the progression of Alzheimer Disease;

- **In several neurobehavioural parameters (MWM Thigmotaxis, Latency and Platform Finding) the treatment groups show no differences if compared to wild-type mice**;

- IACVITA-treated mice show the ability to understand simple tasks and to interact purposefully with the environment, unlike TG mice;

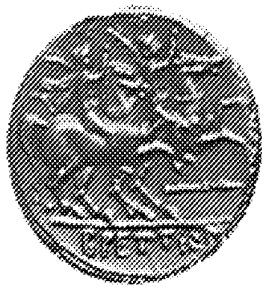
- IACVITA-treated mice show a significant decrease in plaque count in ventral cortex, and a positive trend in the hippocampus;

Results will be published in 2009

IAC project

SUMMARY OF PRECLINICAL DATA ON STROKE AND BRAIN ISCHEMIA

EXHIBIT 2



University of Catanzaro "Magna Graecia"

Faculty of Pharmacy

The protective effect of IAC on BCCO post-ischemic brain damage in Mongolian gerbils.

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

- Male Mongolian Gerbils (total N=66).
- 5 min bilateral common carotid artery occlusion (BCCO).
- 3 doses: 1, 5 or 10 mg/kg i.p. (single)
- Time window: 1 hour before, 1 or 6 hours after the onset of BCCO.
- Evaluation of ultrastructural and neuropathological changes occuring within the hippocampal CA1 area.

MEDESTEA
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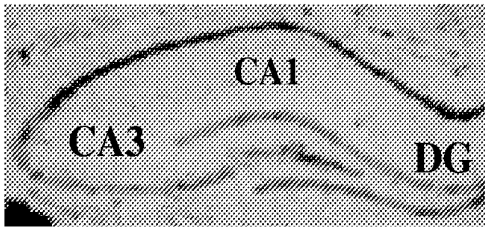
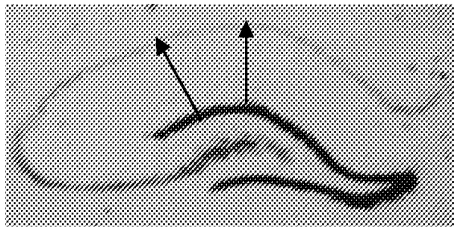
Administration of 10 mg/Kg ip of IAC produced a significant reduction of neuropathological and ultrastructural changes within the hippocampus of gerbils.





SHAM **ISCHEMIA** **ISCHEMIA +IAC**

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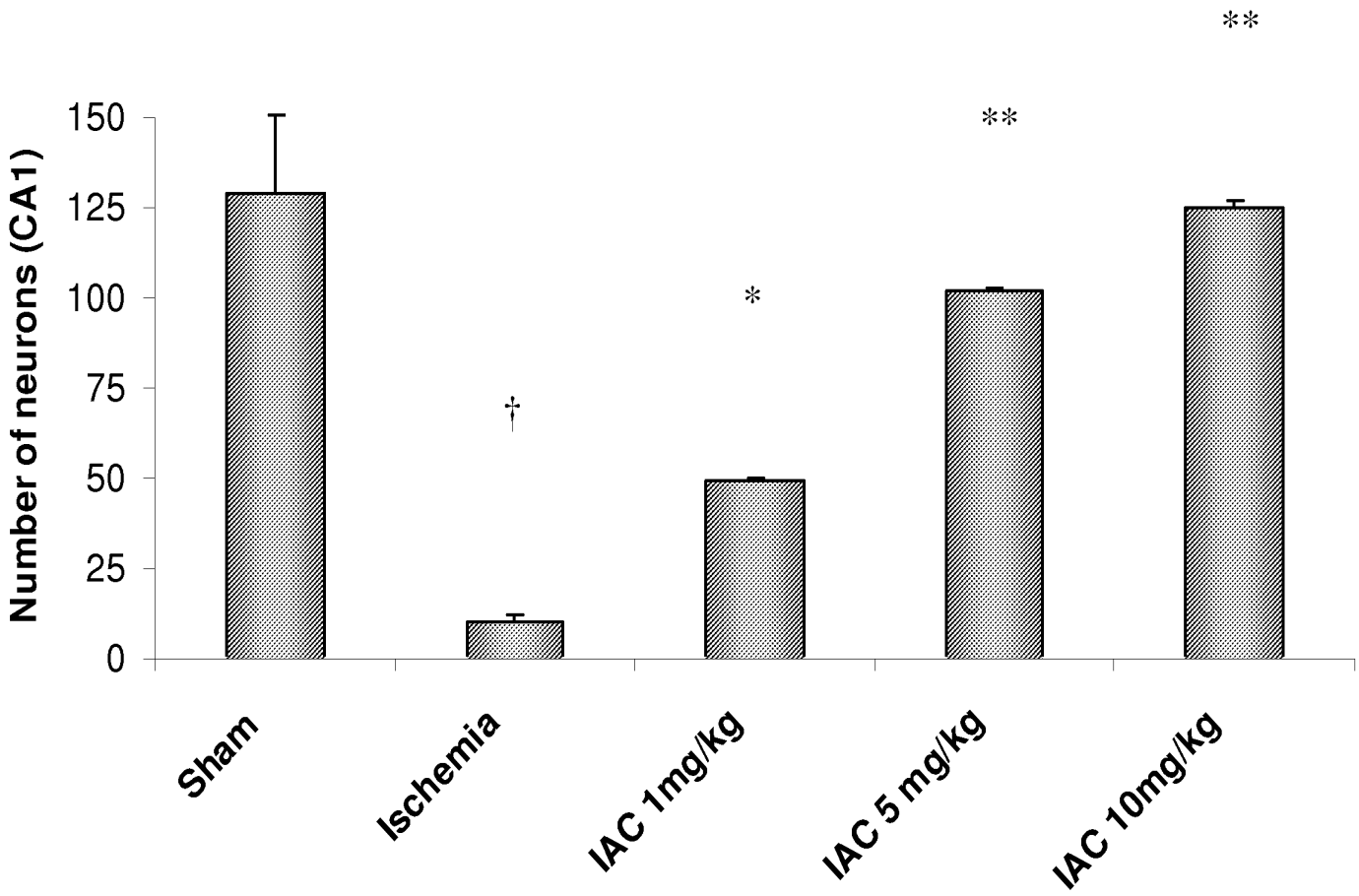
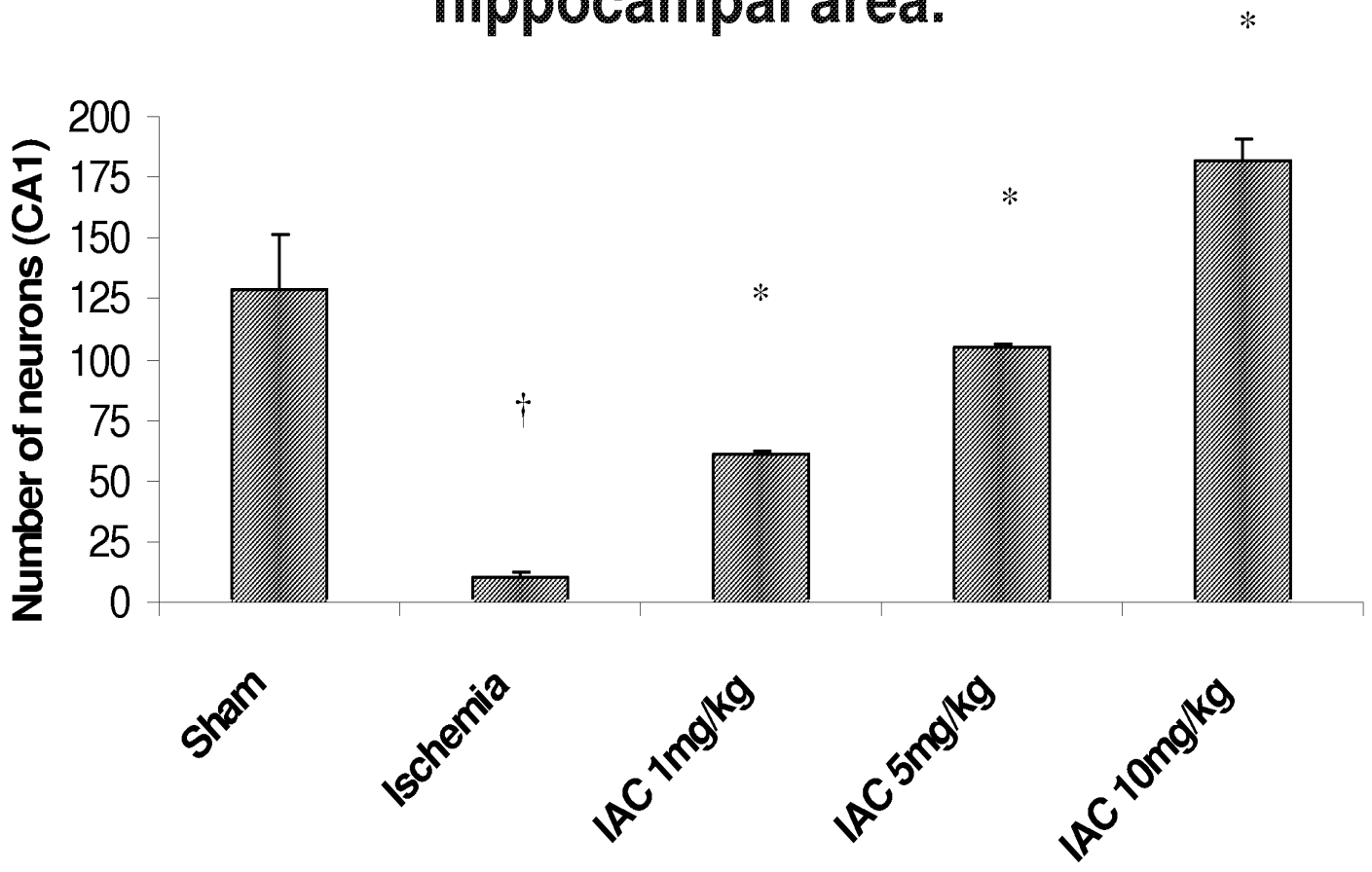
IAC administrated ip 1 hour before BCCO showed a dose dependent protective effect against ischemia-reperfusion induced reduction of neuronal cell number in CA1 hippocampal area.



MEDESTEA
RESEARCH & PRODUCTION S.r.l.

IAC administrated ip 1 hour after BCCO showed a dose dependent protective effect against ischemia-reperfusion induced reduction of neuronal cell number in CA1 hippocampal area.



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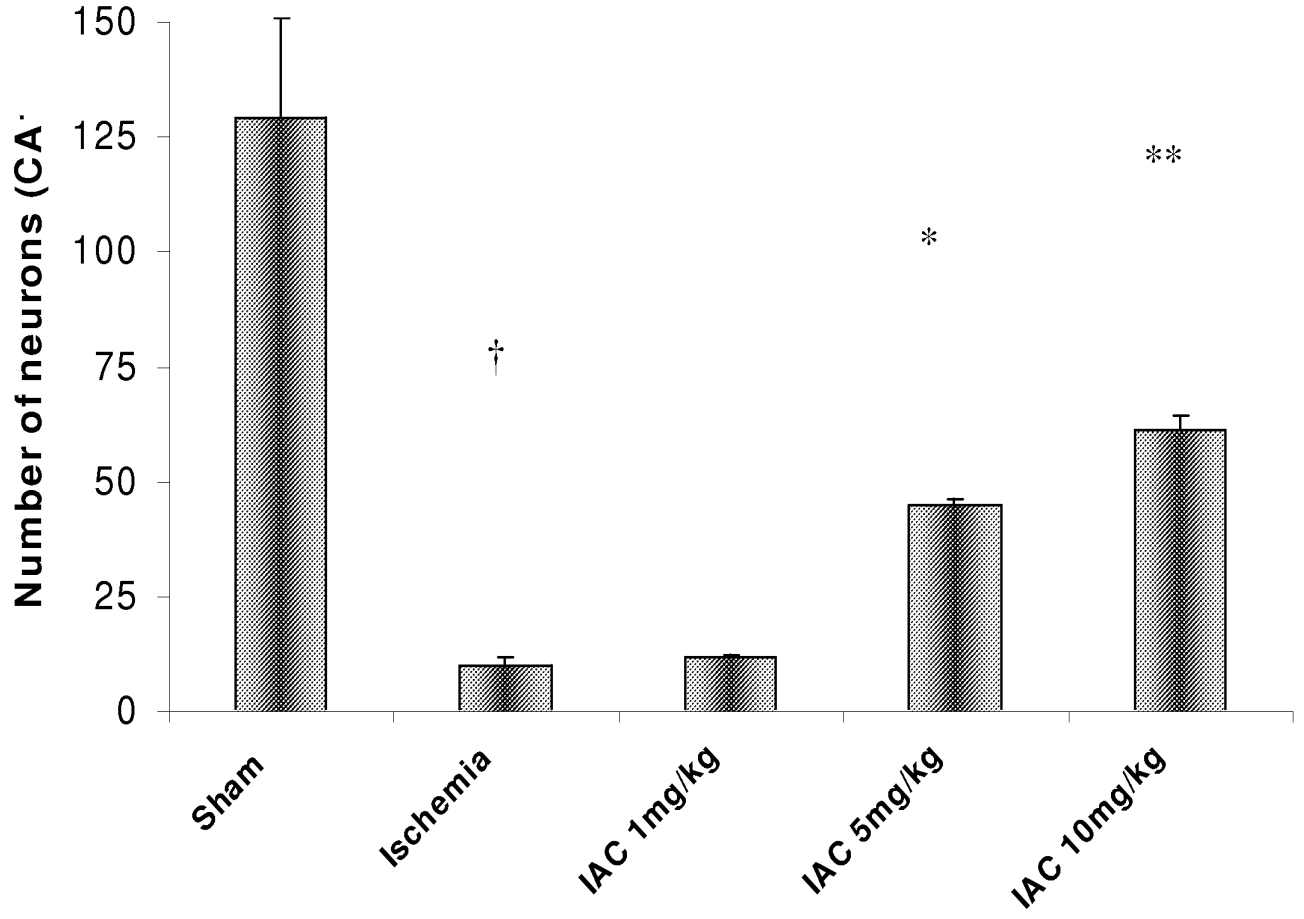
IAC administrated ip 6 hour after BCCO showed a significant protective effect against ischemia-reperfusion induced reduction of neuronal cell number in CA1 hippocampal area at a dose of 5 and 10mg/ml.



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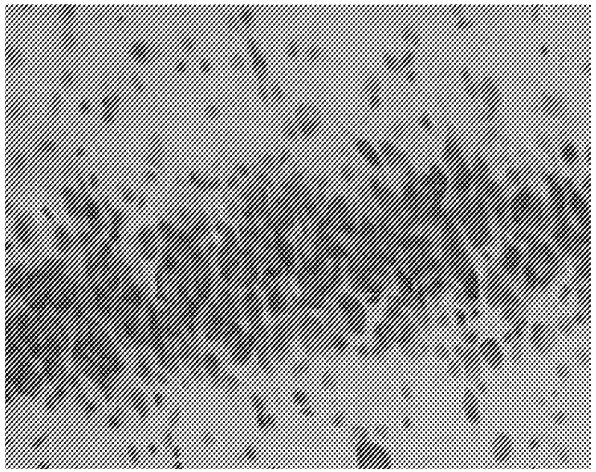
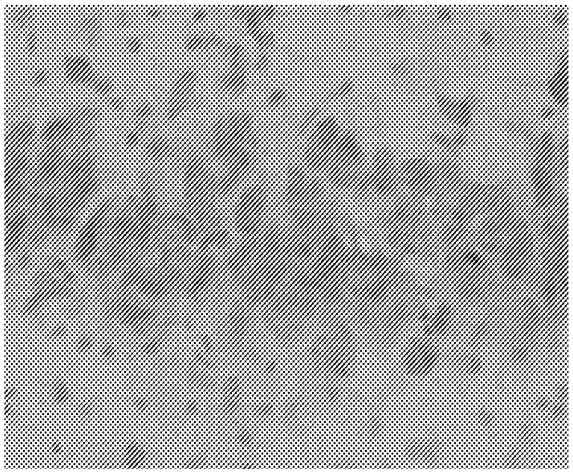
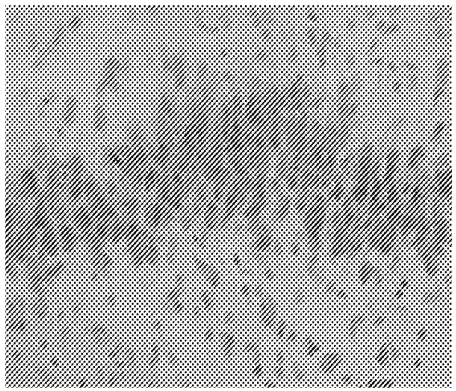
i.p. administration of IAC protected against ischemia-reperfusion hippocampal early lesion.



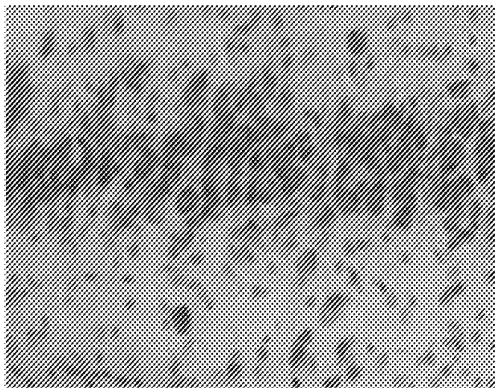
Sham



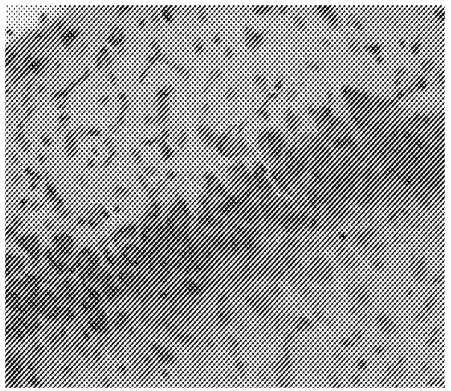
Ischemia



IAC 1mg



IAC 5mg



IAC 10mg

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CONCLUSIONS

1) It is known that ischemia-reperfusion of brain tissue is followed by an increased production of reactive oxygen species (ROS) which, in turn, participate in the mechanisms leading to post-ischemic neuronal cell death.

2) The present experiments demonstrate that IAC, the most powerful safe free radical scavenger known today, produces a relevant and significant protective effect against neuropathological changes elicited by temporary BCCO, even if administrated 1 to 6 hours after the ischemic damage.

3) The present experiments indicate also that IAC quickly reaches concentrations in the brain able to develop a strong pharmacological activity when administrated peripherally.

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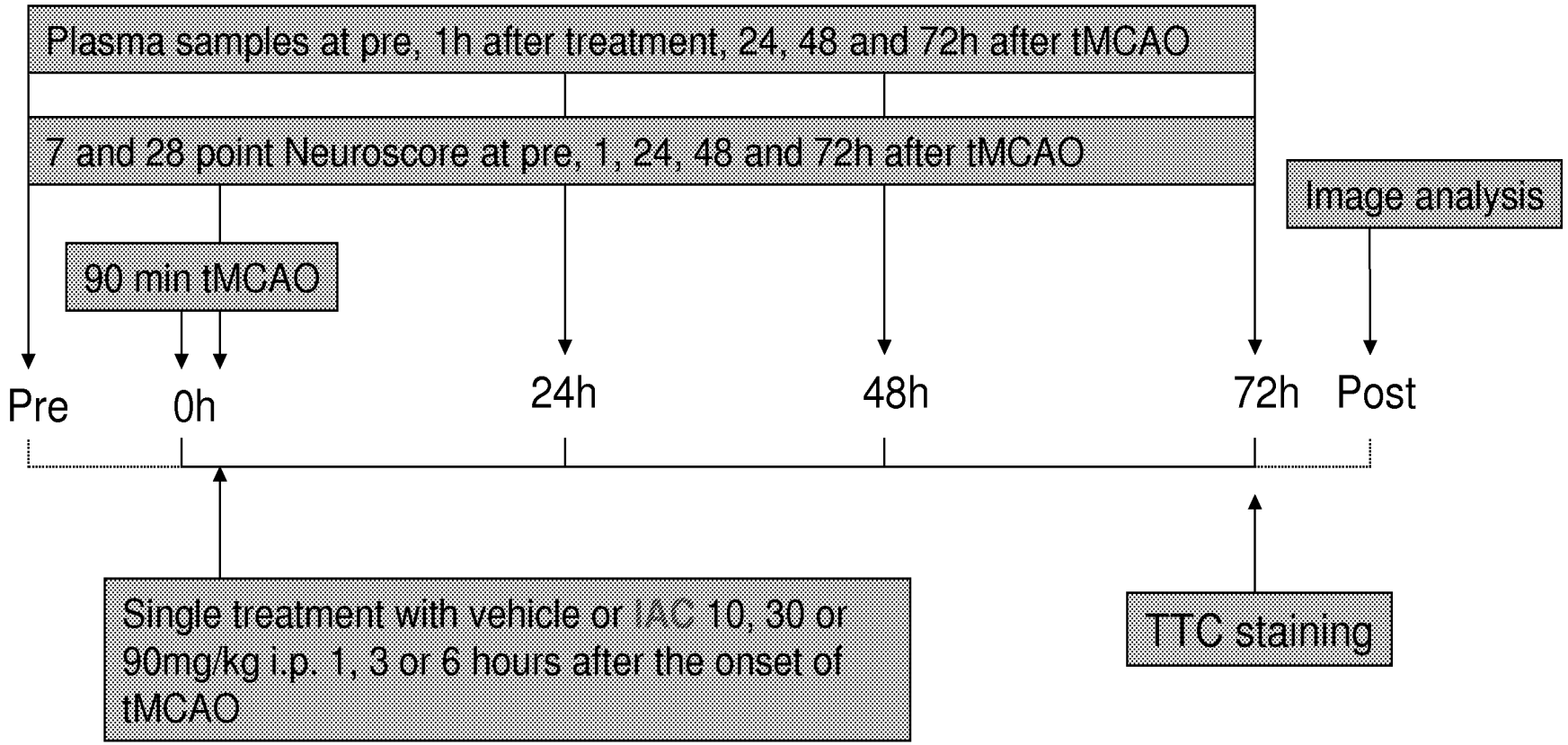
*Effect of i.p. **IAC** treatment on Infarct Volume and Sensory-Motor Behaviour in tMCAO rats*

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

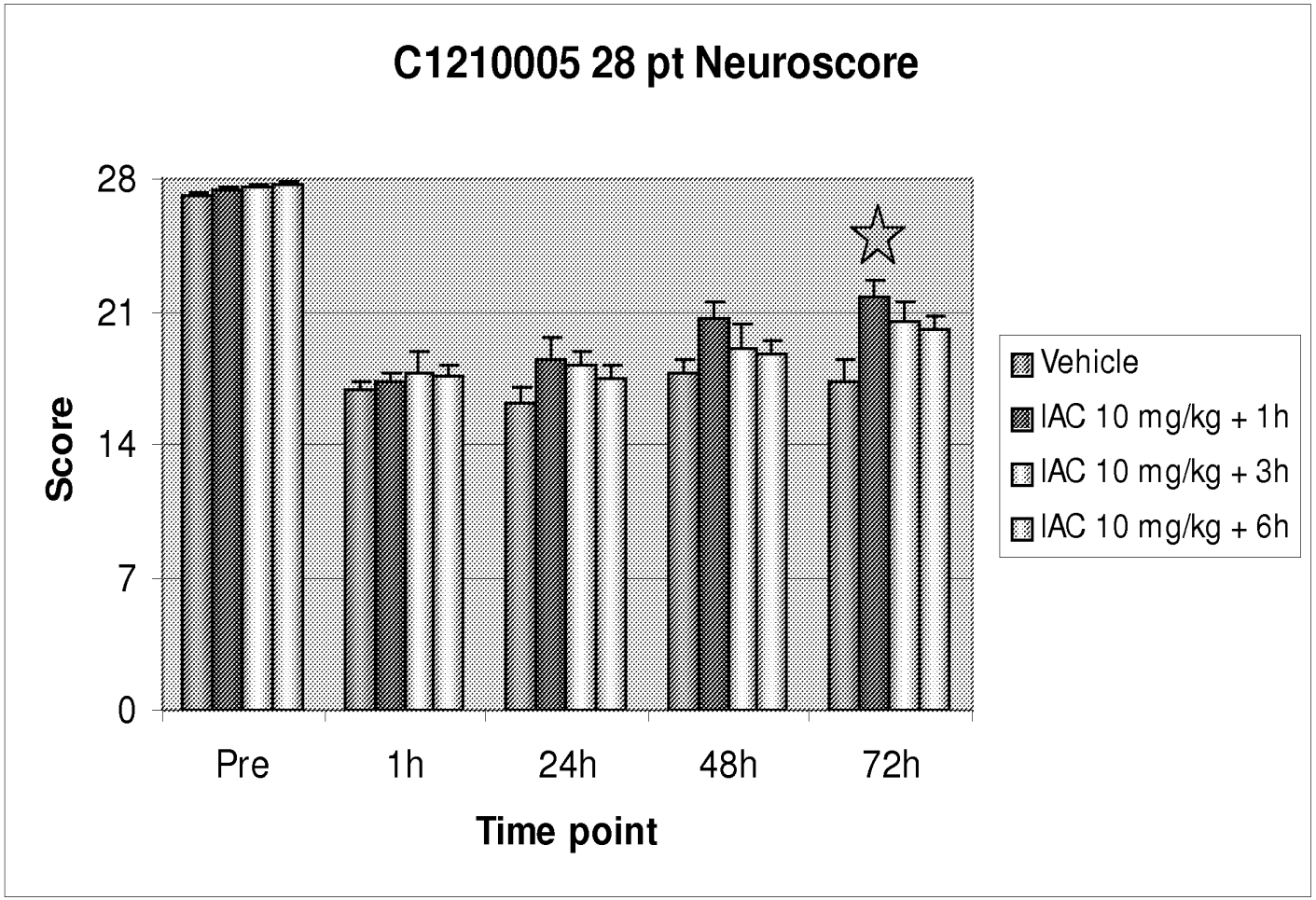
- Male Sprague-Dawley rats (total N=120)
- 90 min transient focal cerebral ischemia (tMCAO)
- 3 doses: 10, 30 or 90 mg/kg i.p. (single)
- Time window: 1, 3 or 6 hours after the onset of tMCAO
- Evaluation of sensory-motor performance: 7 and 28 point Neuroscore
- Evaluation of brain damage: total, cortical and subcortical infarct volume

Schematic of study protocol



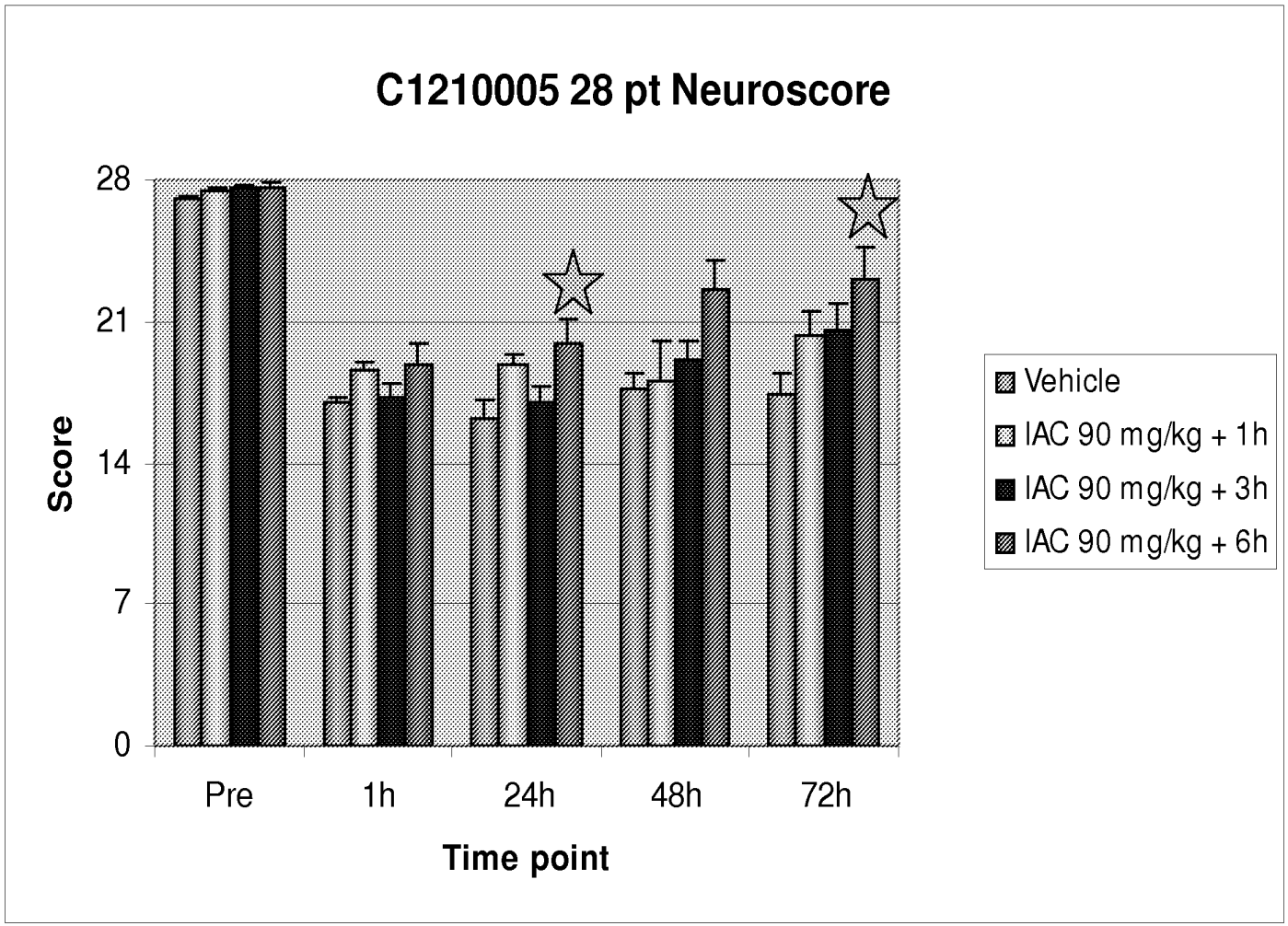
Results

28 point Neuroscore (IAC 10 mg/kg)



Results

28 point Neuroscore (IAC 90 mg/kg)



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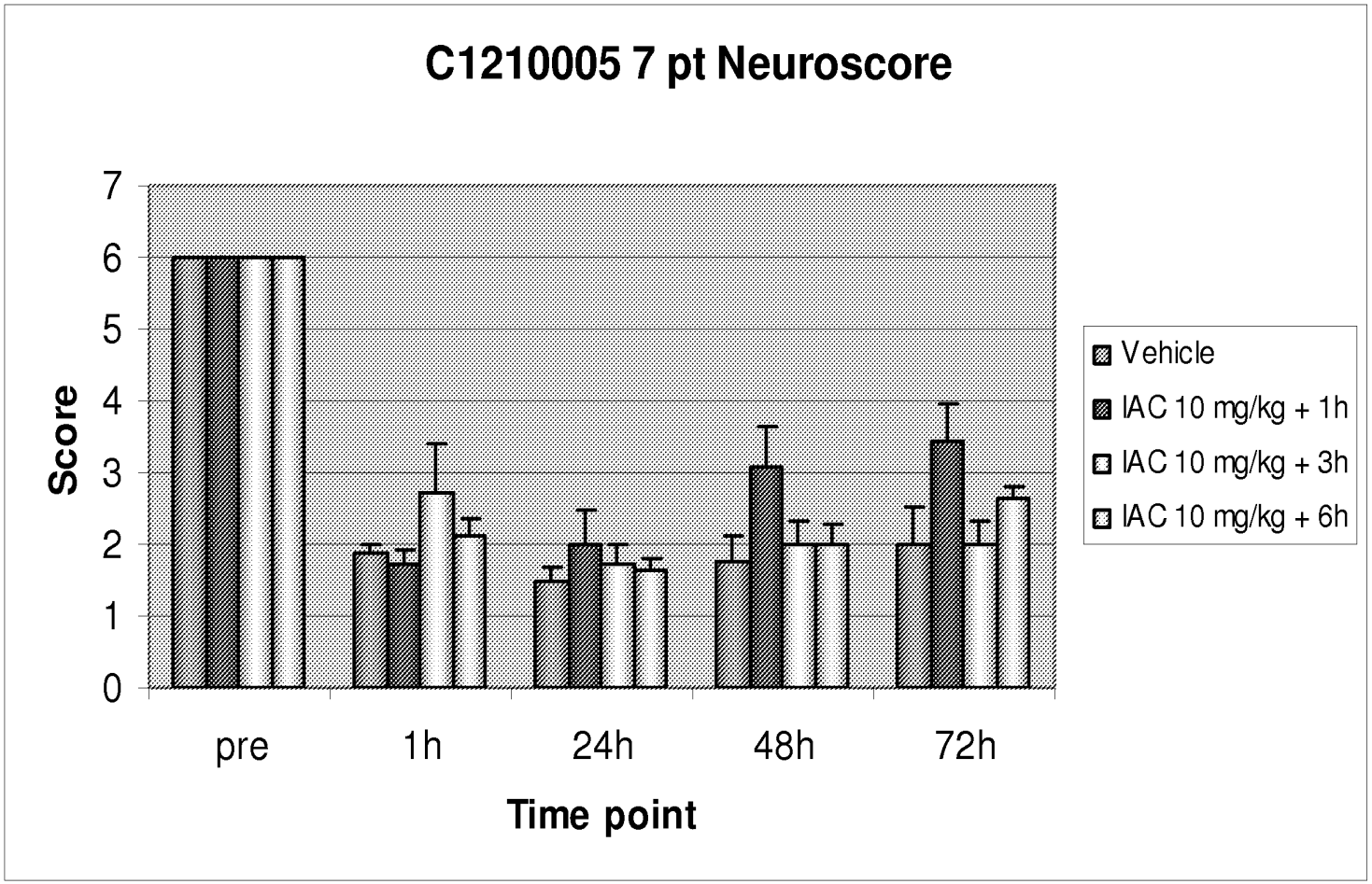
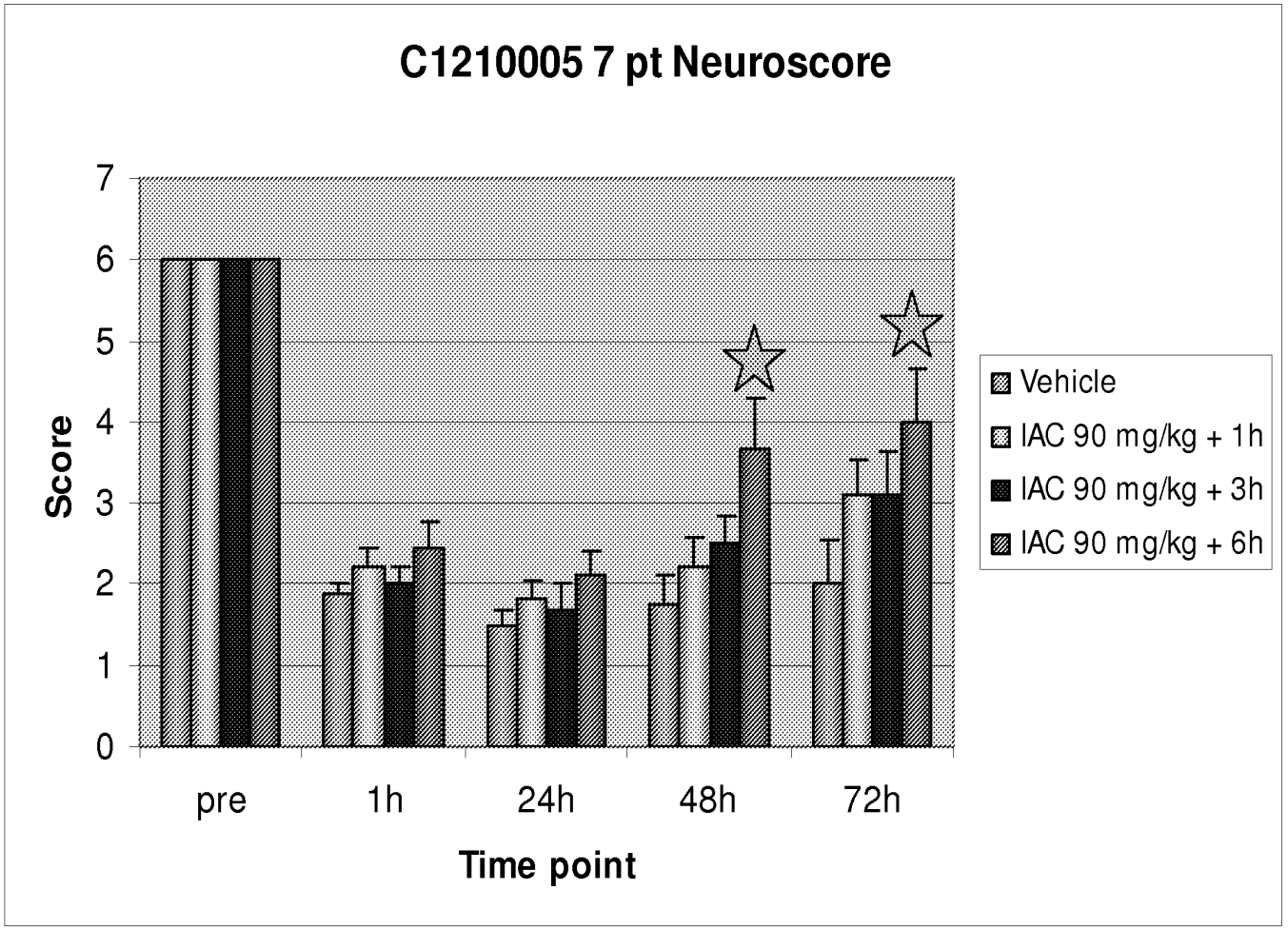
Results

7 point Neuroscore (IAC 10 mg/kg)



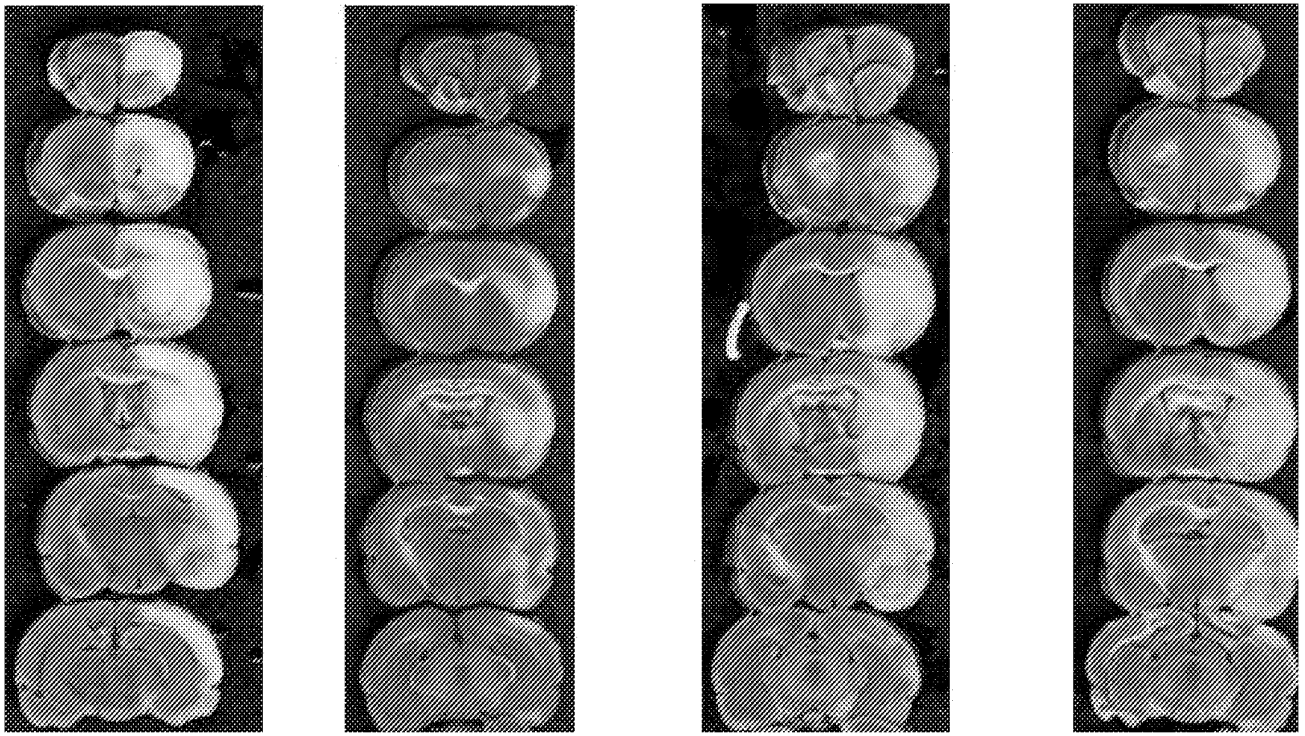
Results

7 point Neuroscore (IAC 90 mg/kg)



Results

Infarct volume (IAC 10 mg/kg)



Vehicle
Rat# 1

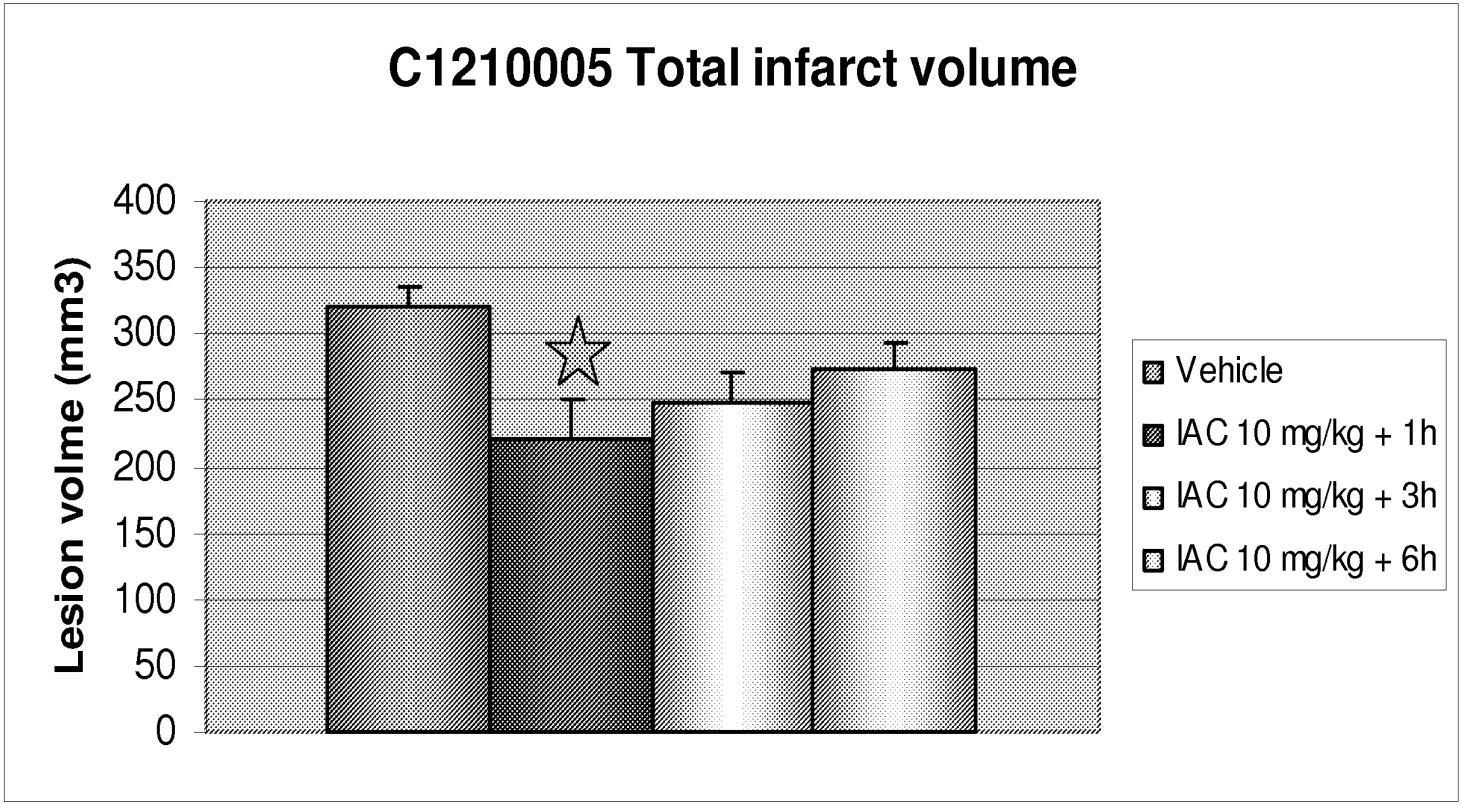
10mg/kg (1h)
Rat# 13

10mg/kg (3h)
Rat# 71

10mg/kg (6h)
Rat# 106

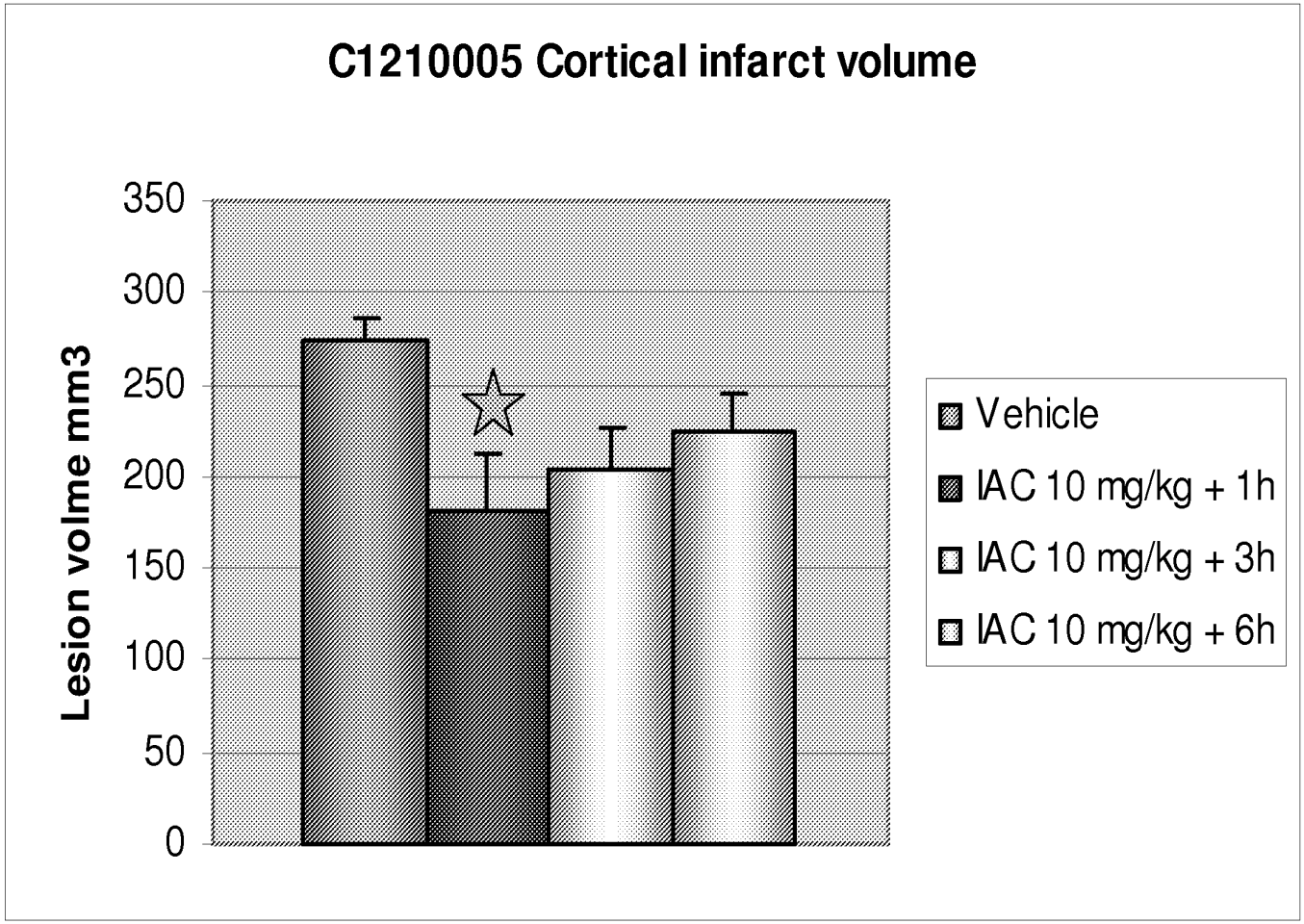
Results

Total infarct volume (IAC 10 mg/kg)



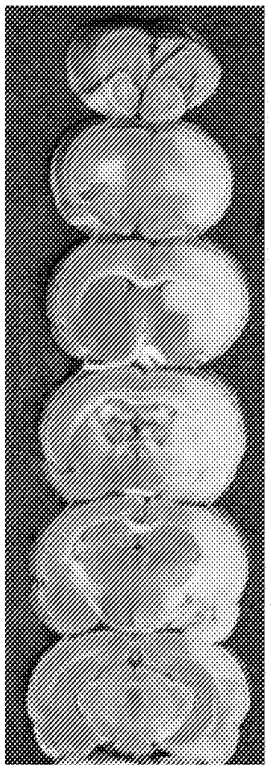
Results

Cortical infarct volume (IAC 10 mg/kg)

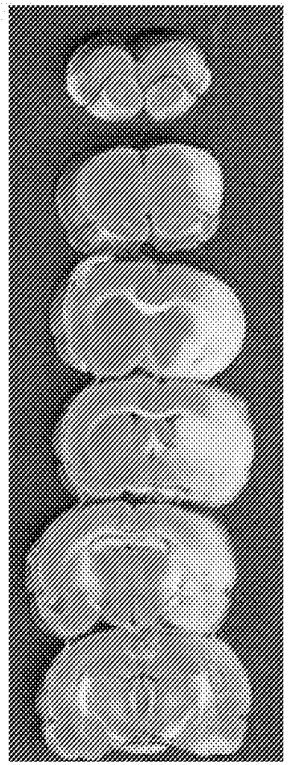


Results

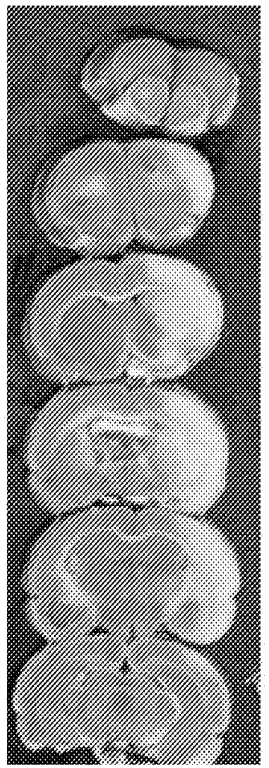
Infarct volume (IAC 90 mg/kg)



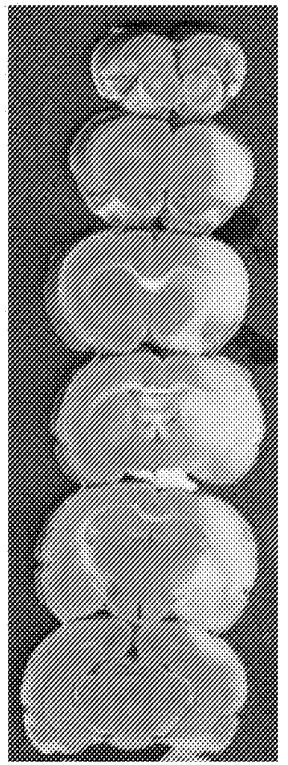
Vehicle
Rat# 86



90mg/kg (1h)
Rat#44



90mg/kg (3h)
Rat # 38



90mg/kg (6h)
Rat# 92

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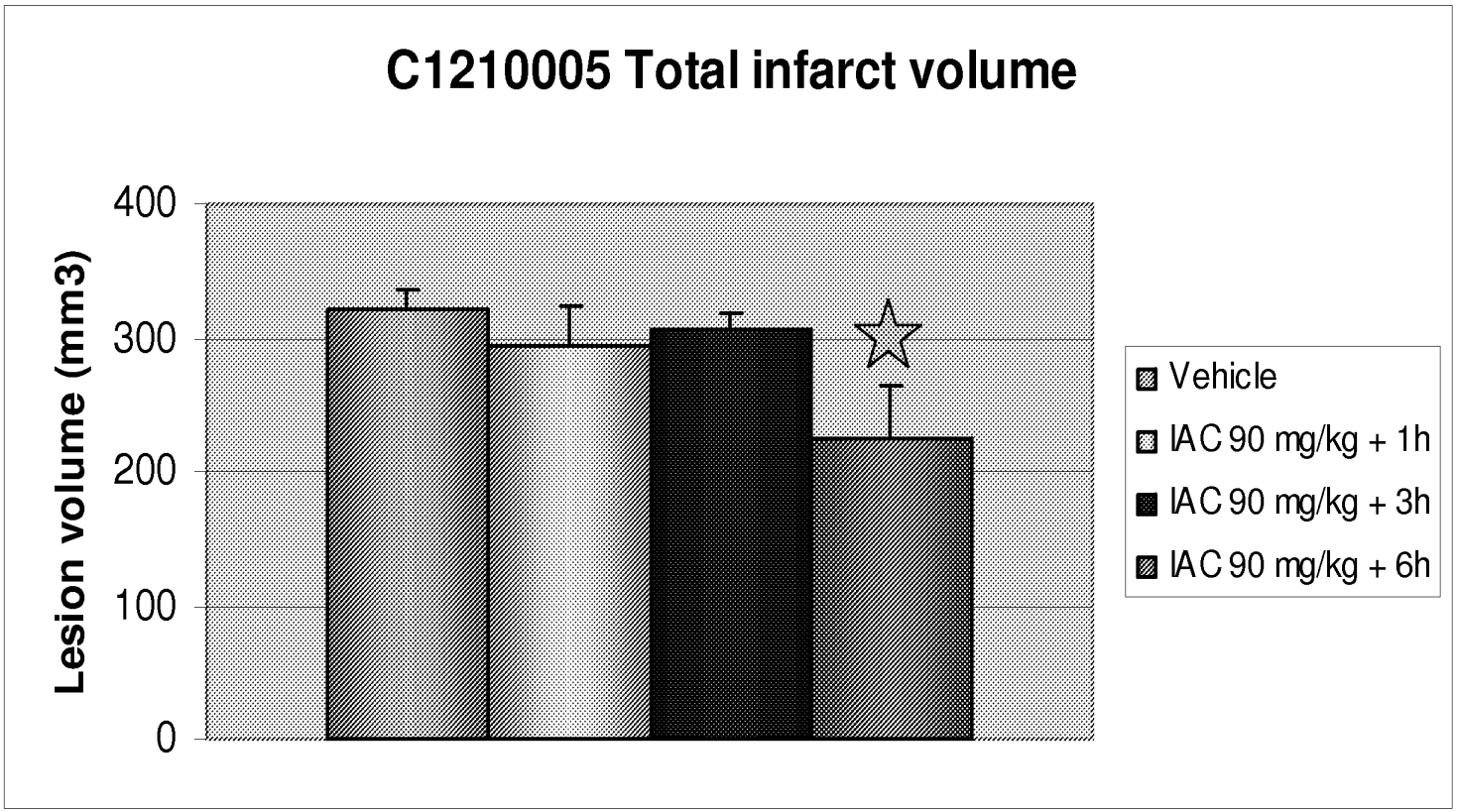
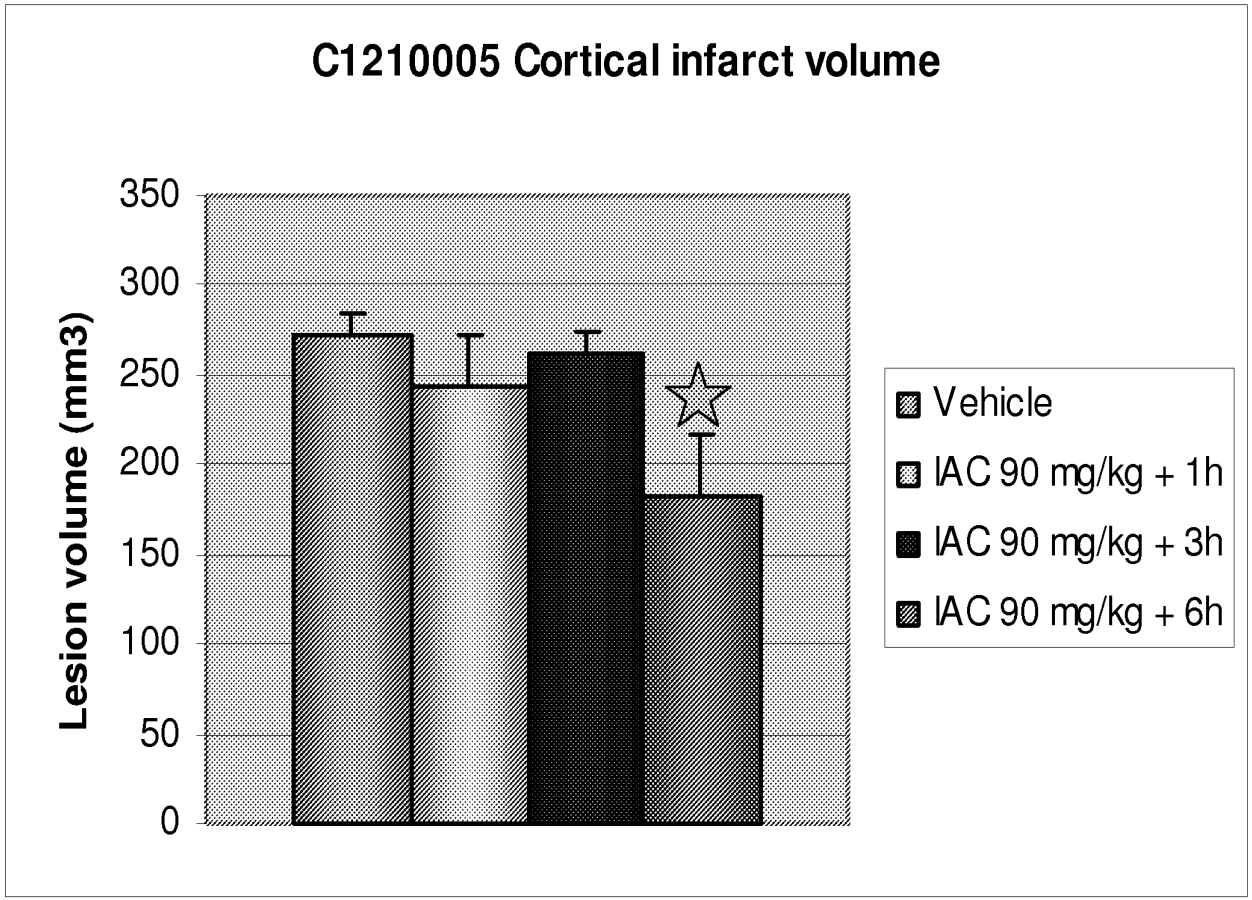
Results

Total infarct volume (IAC 90 mg/kg)



Results

Cortical infarct volume (IAC 90 mg/kg)



Conclusions

1) The present experiment demonstrates that IAC produces a relevant and significant protective effect against neuropathological changes elicited and by tMCAO in rats:

- At the dose of 10 mg/kg, 1 hour after the tMCAO onset;
- At the dose of 90 mg/kg, 6 hours after the tMCAO onset.

2) The results of the current studies indicate also that IAC administrated several hours after the onset of the tMCAO, provides significant improvement of long term functional recovery.

3) The present experiment indicates also that IAC quickly reaches concentrations in the brain able to develop a strong pharmacological activity when administrated peripherally and can elicit its activity by i.p. administration.

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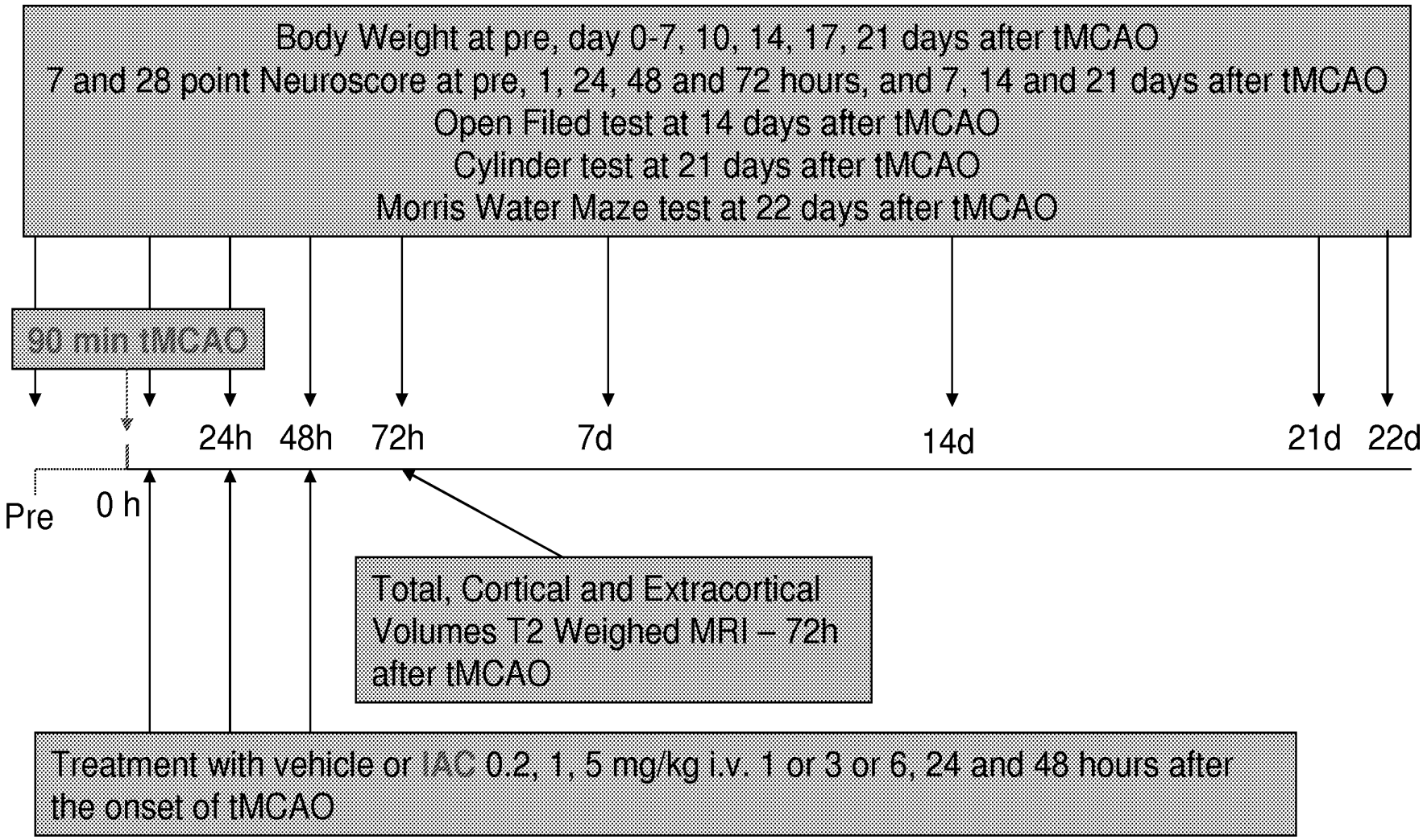
*Effect of i.v. **IAC** treatment on Infarct Volume and Sensory-Motor Behaviour in tMCAO rats*

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

- Male Sprague-Dawley rats (total N=120)
- 90 min transient focal cerebral ischemia (tMCAO)
- 3 doses: 0.2, 1, 5 mg/kg i.v.
- Time window: 1 or 3 or 6, 24 and 48 hours after the onset of tMCAO
- Evaluation of sensory-motor performance: 7 and 28 point Neuroscore, Cylinder test, Open Field test, Morris Water Maze test
- Evaluation of brain damage: total, cortical and subcortical infarct volume

Schematic of study protocol



MEDESTEA
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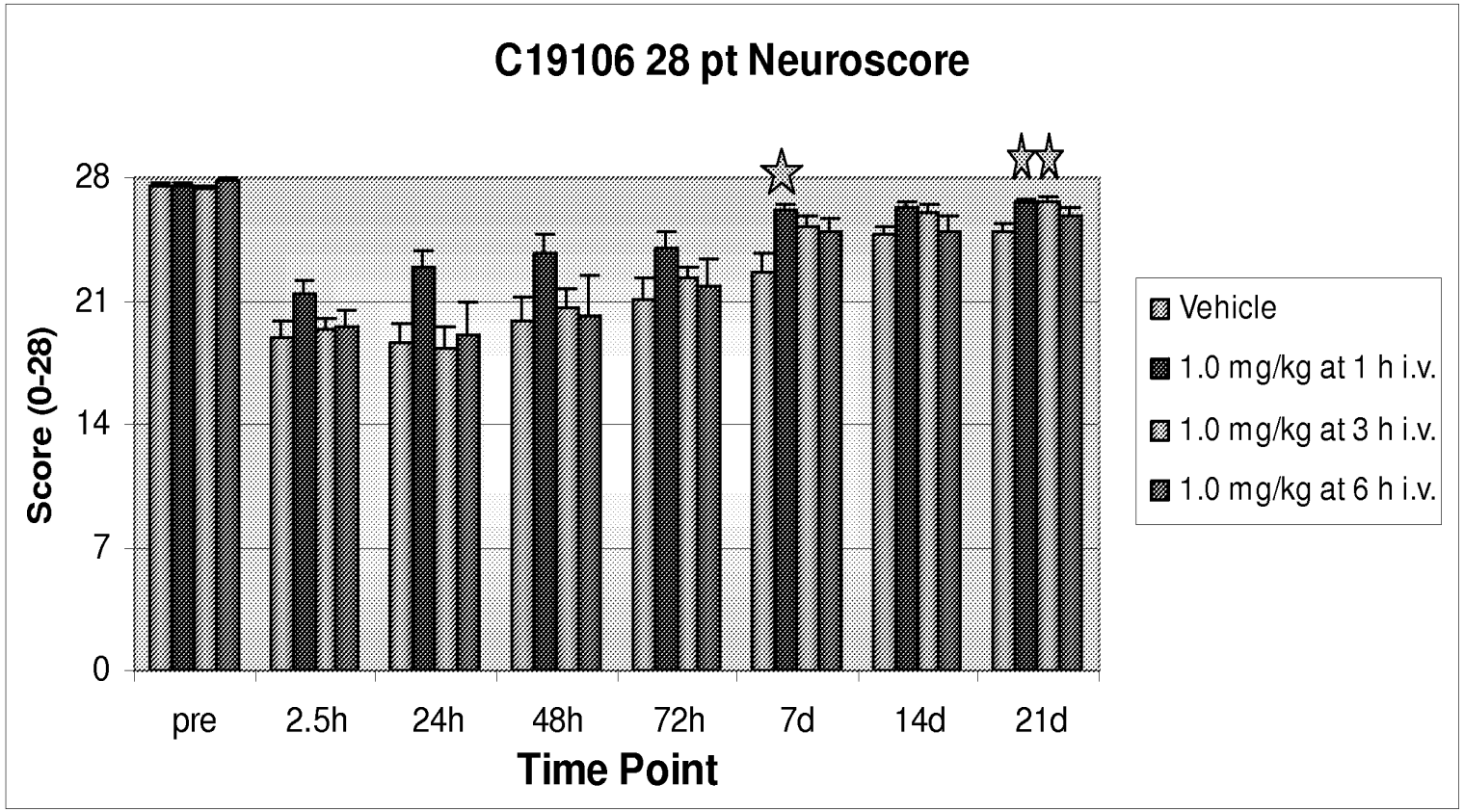
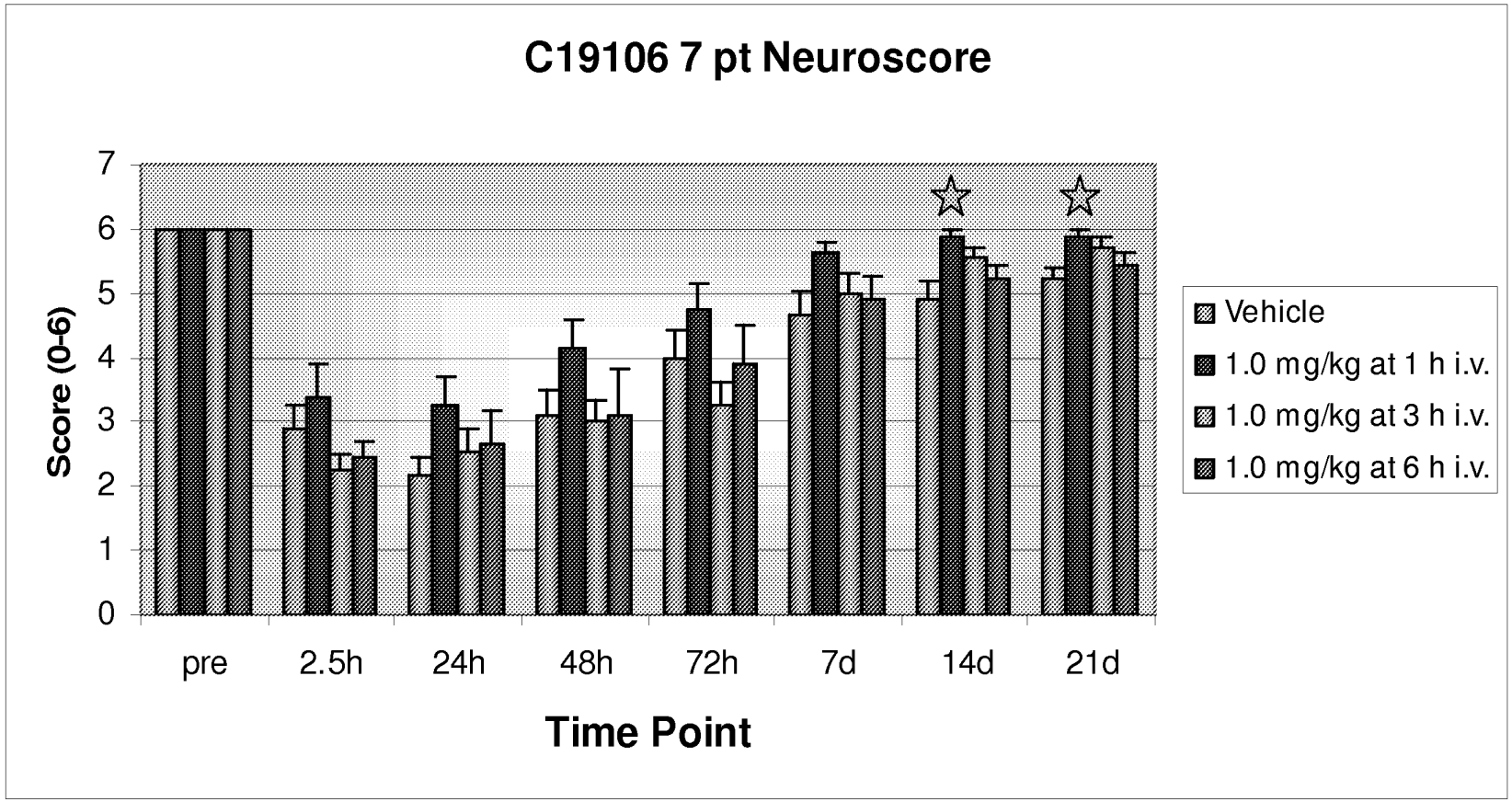
Results

28 point Neuroscore (IAC 1 mg/kg)



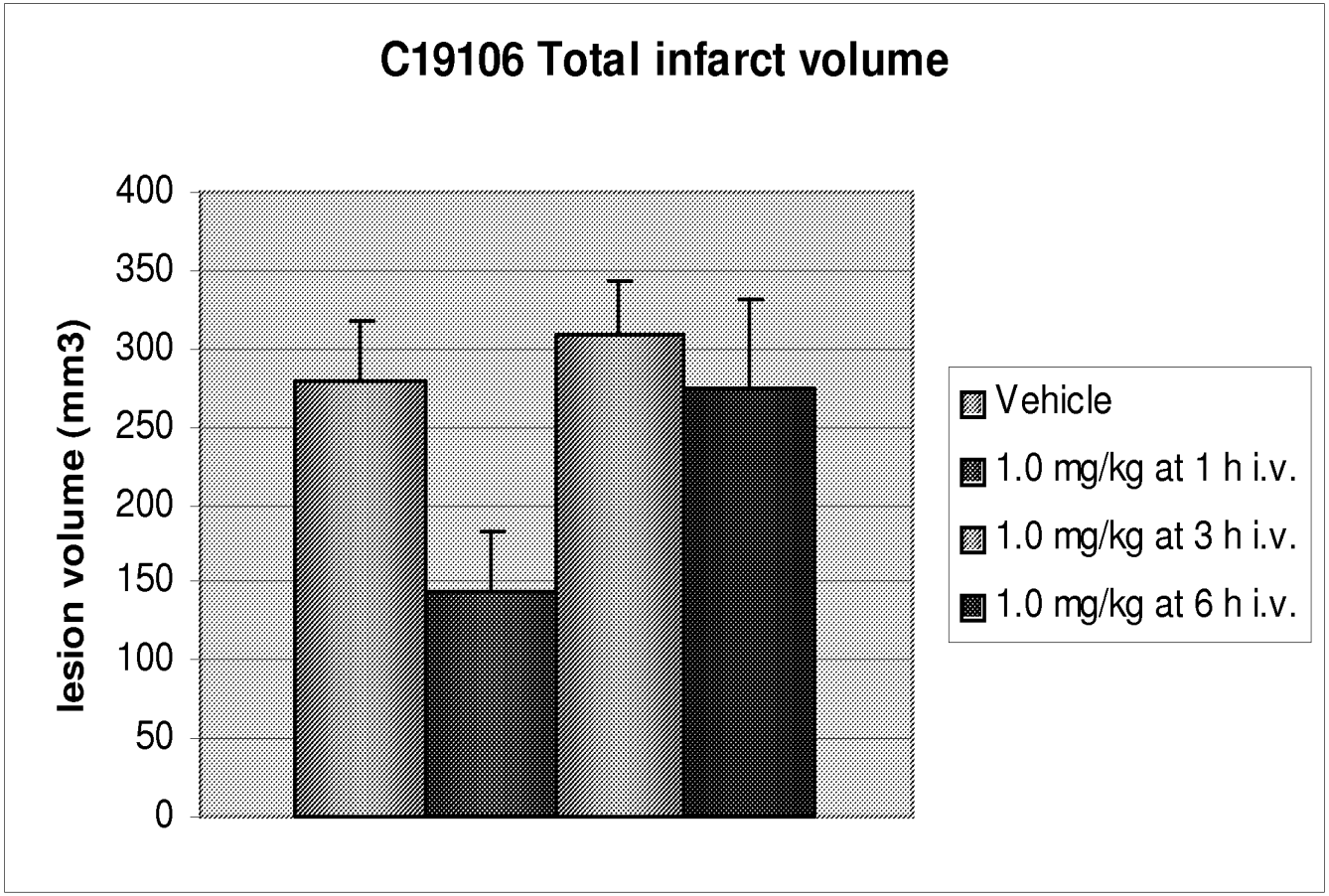
Results

7 point Neuroscore (IAC 1 mg/kg)



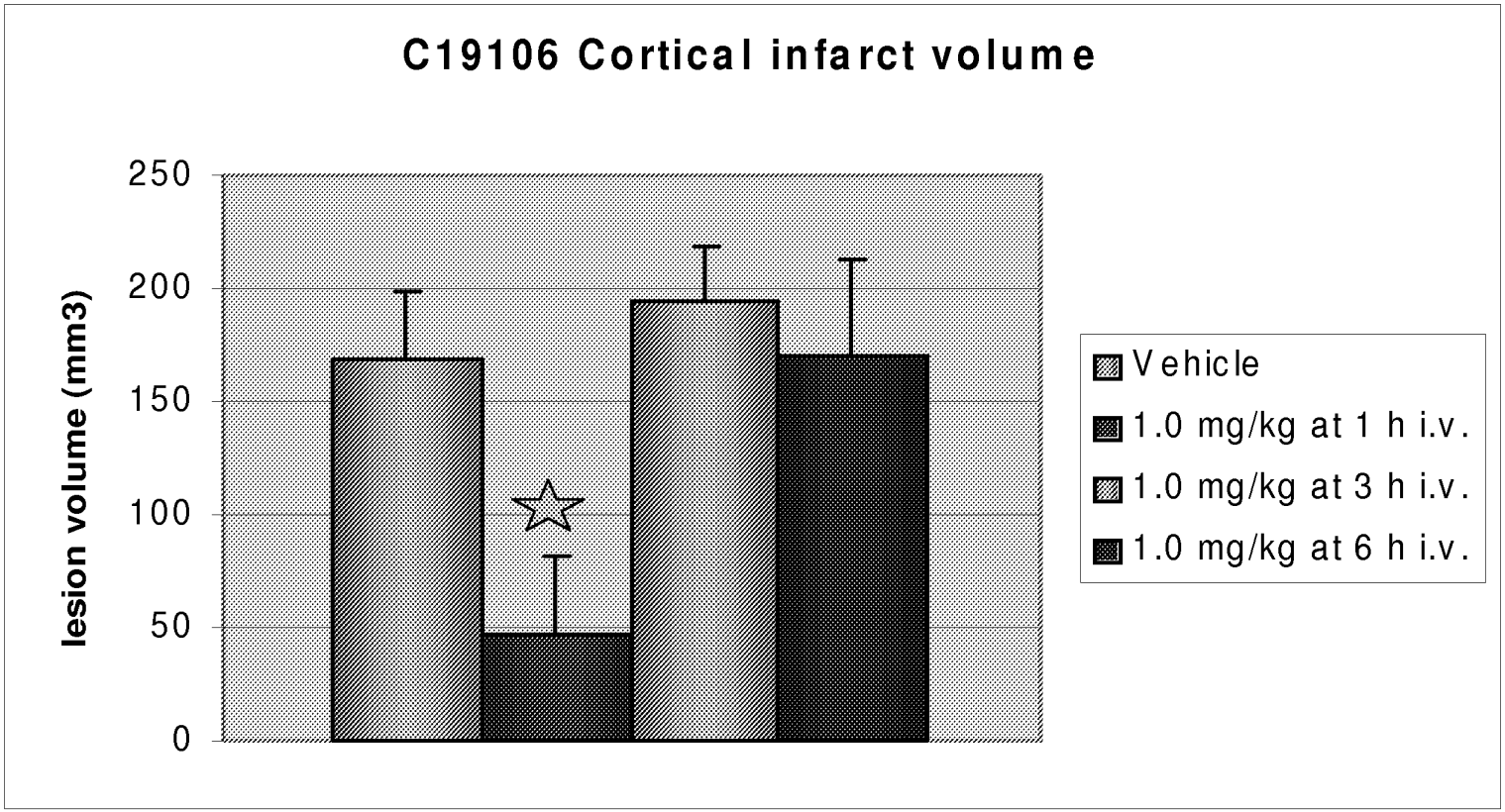
Results

Total infarct volume (IAC 1 mg/kg)



Results

Cortical infarct volume (IAC 1 mg/kg)



Conclusions

1) The present experiments demonstrate that IAC produces a relevant and significant protective effect against neuropathological changes elicited by tMCAO in rats, even if administrated several hours after the ischemic damage.

2) The results of the current studies indicate also that IAC administrated several hours after the onset of the tMCAO, provides significant improvement of long term functional recovery.

3) The present experiments indicate also that IAC quickly reaches concentrations in the brain able to develop a strong pharmacological activity when administrated peripherally and can elicit its activity by i.v. administration.

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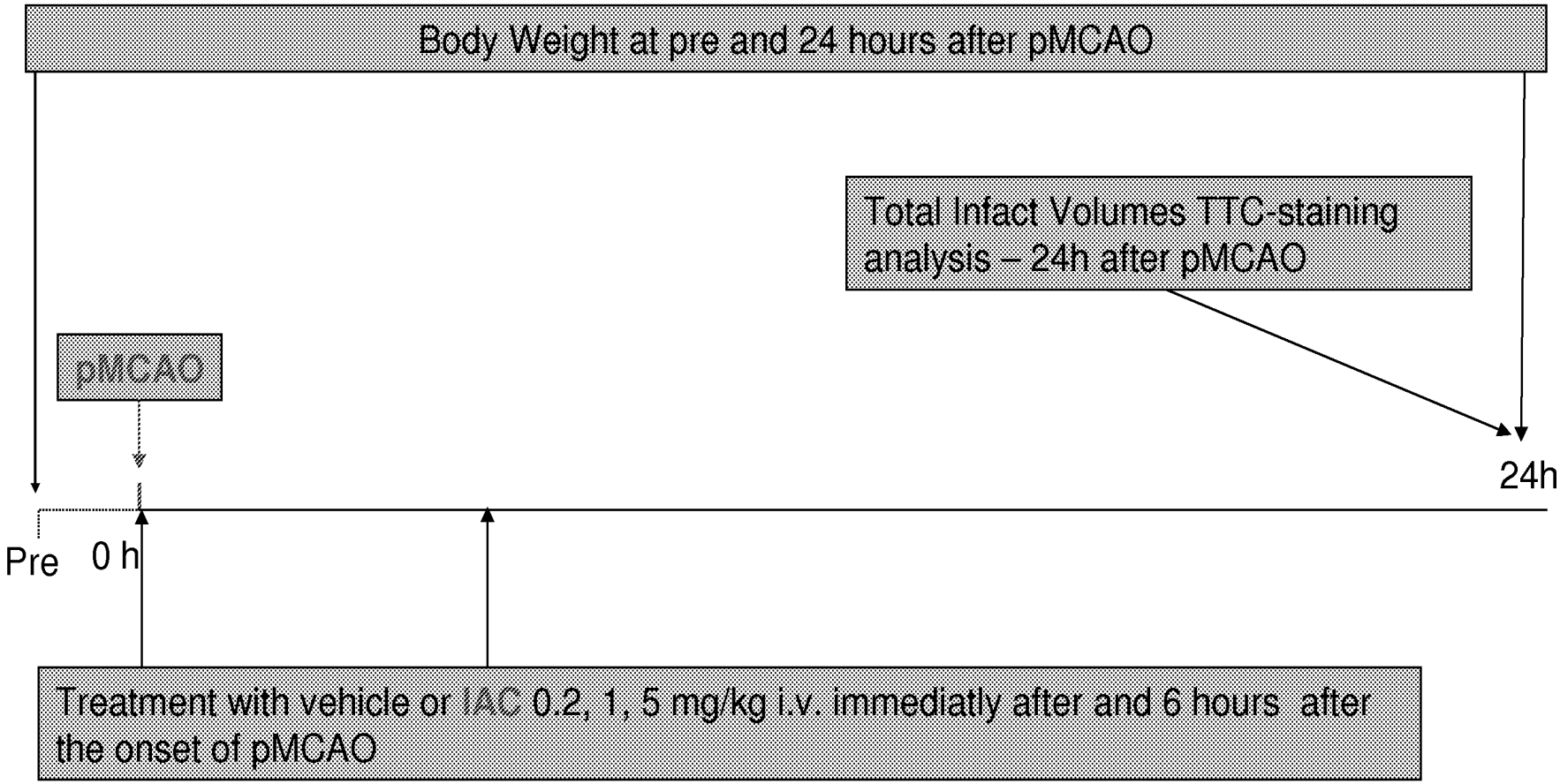
*Effect of i.v. **IAC** treatment on Infarct Volume in pMCAO mice*

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

- Male C57Bl/6 mice (total N=50)
- Permanent focal cerebral ischemia (pMCAO)
- 4 doses: 0.2, 1, 5, 10 mg/kg i.v.
- Time window: immediatly after and 6 hours after the onset of pMCAO
- Evaluation of brain damage: total, cortical and subcortical infarct volume

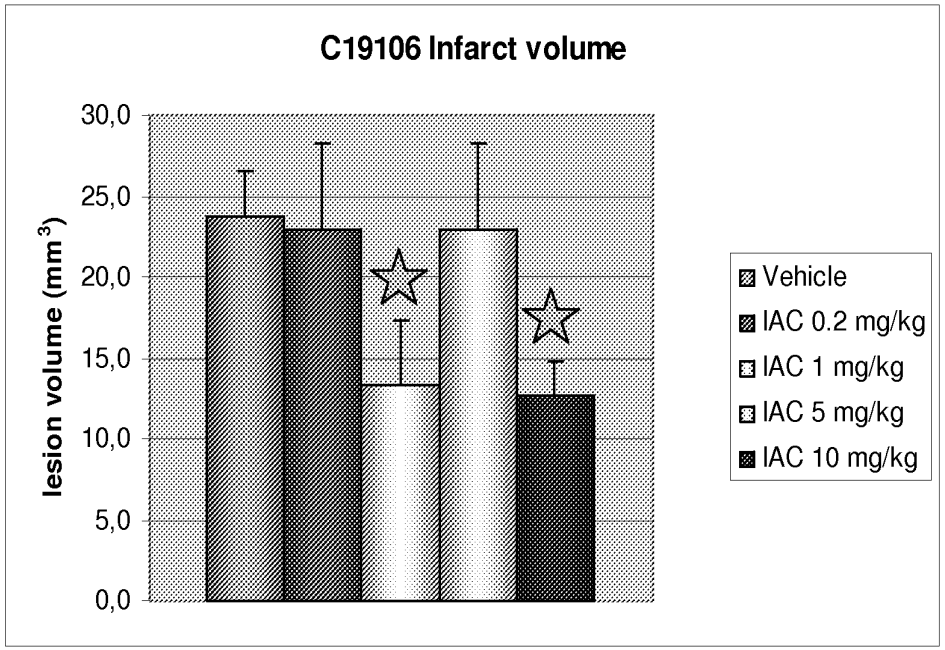
Schematic of study protocol



Results

Infarct Volume at 24 h after pMCAO

Group	N° of animals with 0-lesion
Vehicle	0/10
IAC 0.2 mg/Kg	2/10
IAC 1 mg/Kg	3/10
IAC 5 mg/Kg	2/10
IAC 10 mg/Kg	0/9



Conclusions

1) The present experiment suggests that IAC (1 and 10 mg/Kg) significantly reduces infarct volume at 24 hours after pMCAO in mice.

2) The results of the current studies indicate also that incidence of 0-infarct was limited only to IAC treated groups.

3) The present experiment may reflect neuroprotective properties of IAC after i.v. administration immediately and 6 hours after pMCAO in mice.

CEREBRICON

Cerebricon

A leader in the non-clinical screening of drug candidates against CNS disease targets.

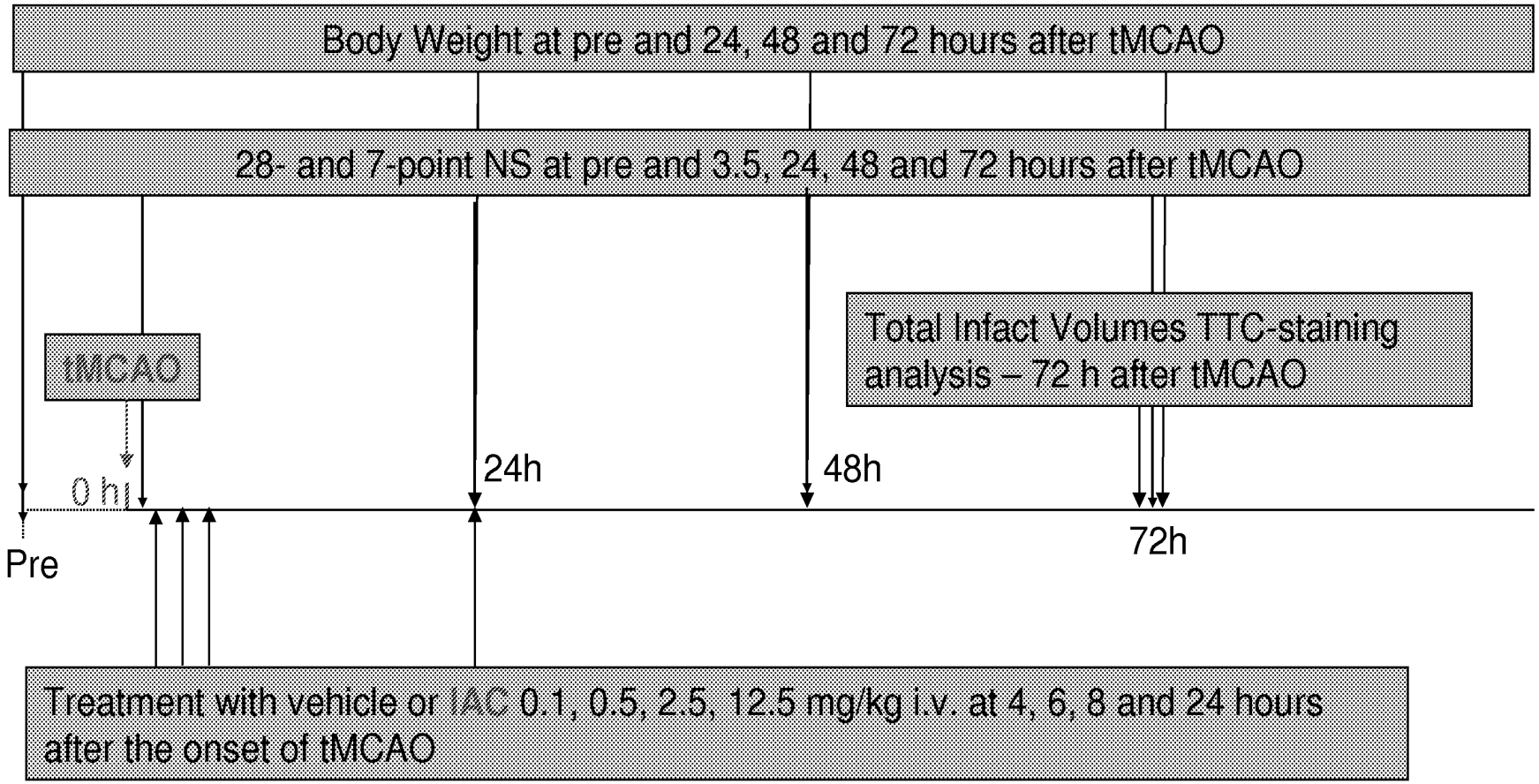
Effect of delayed i.v. ***IAC*** *treatment on Infarct Volume in tMCAO rats*

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

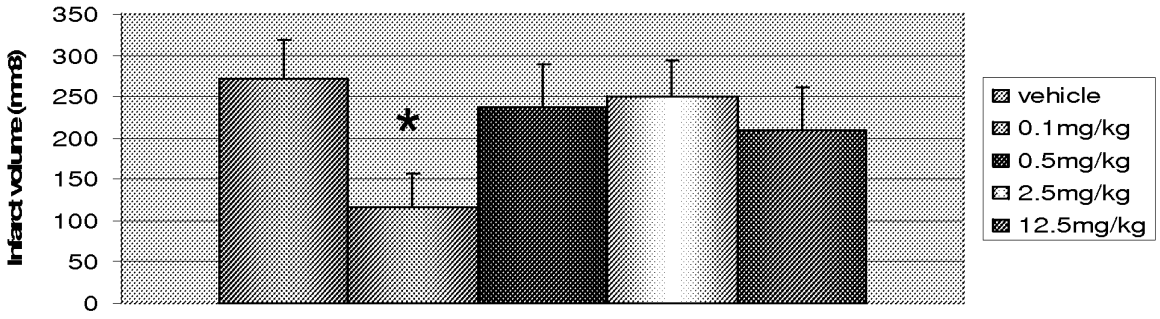
- Male Sprague-Dawley rats (total N=90)
- 90 min middle cerebral artery occlusion (tMCAO)
- 5 doses: 0.1, 0.5, 2.5, 12.5 mg/kg i.v.
- Time window: at 4, 6, 8 and 24 hours after the onset of tMCAO
- Evaluation of:
  - Brain damage (total, cortical and subcortical infarct volume);
  - Sensory-motor behavior (28- and 7-point neuroscore tests)

Study schematics

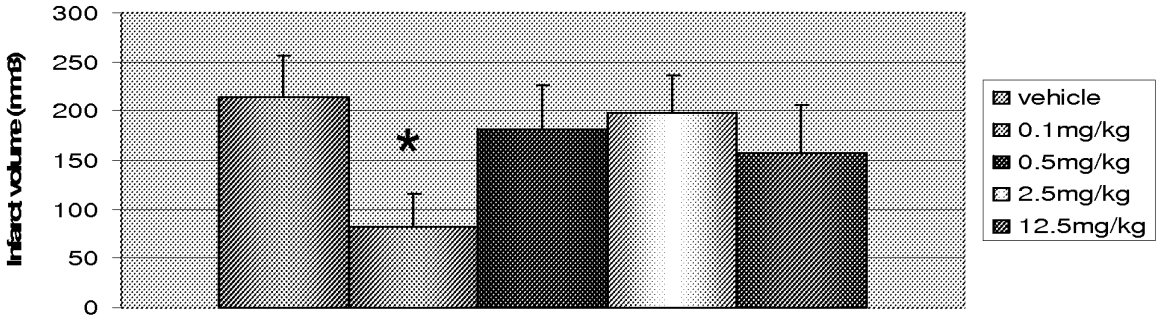


Results

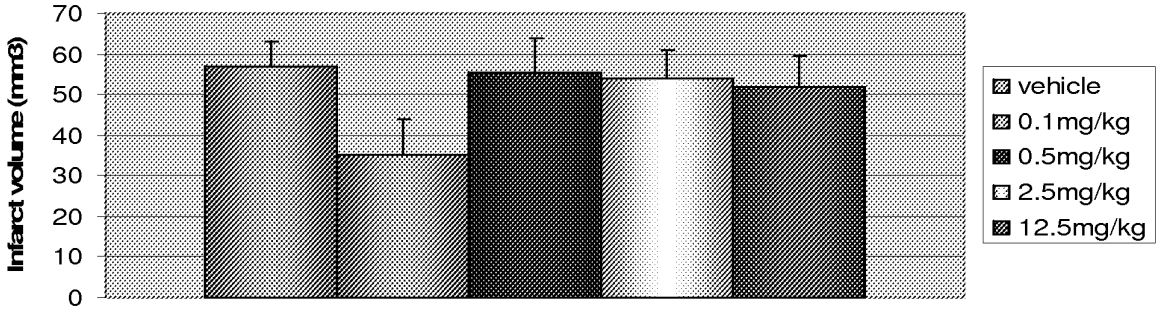
C32006
Total infarct volume



C32006
Cortical infarct volume



C32006
Subcortical infarct volume



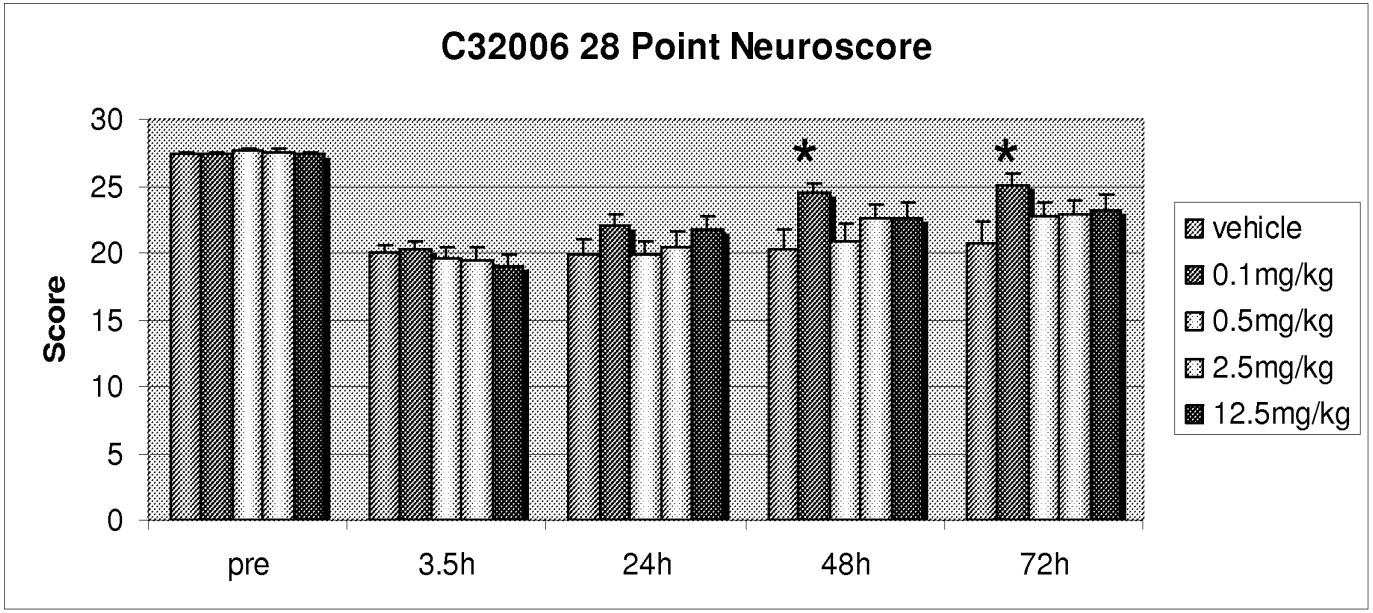
MEDESTEA
RESEARCH & PRODUCTION S.r.l.

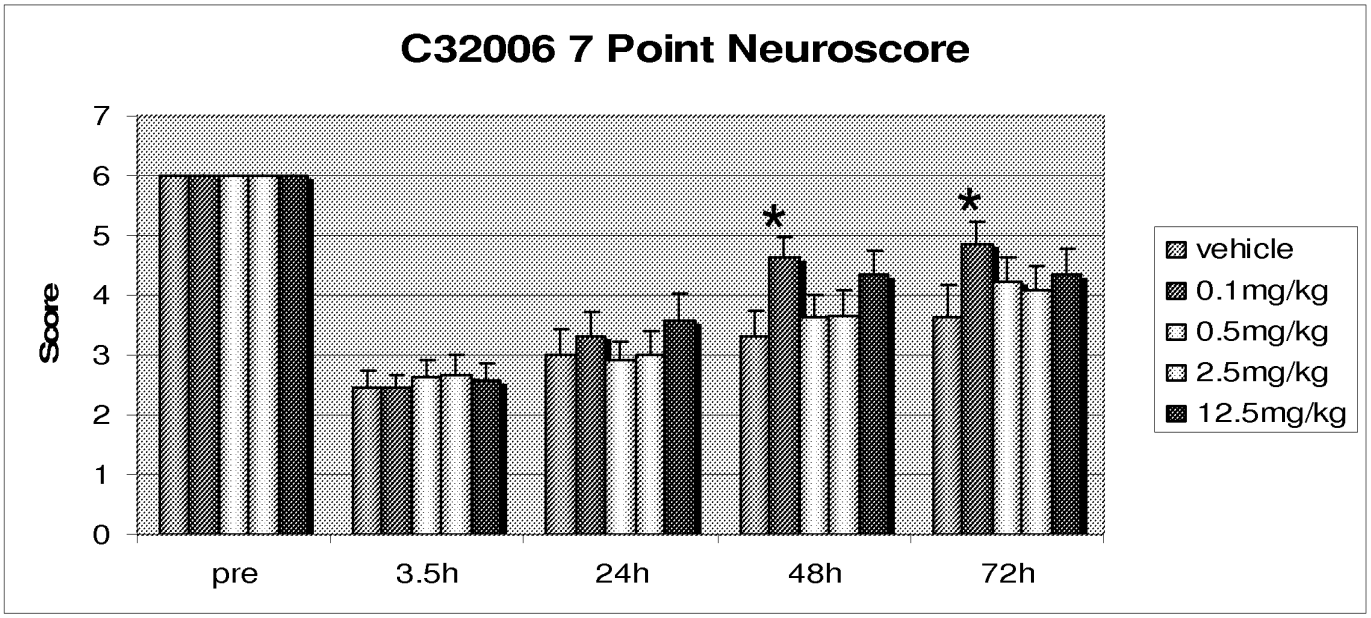
Results

Number of animals with 0-lesions for Total, Cortical and Subcortical Infarct Volume at 72 h after tMCAO

Group	Total	Cortical	Subcortical
Vehicle	1/15	4/15	1/15
IAC 0.1 mg/Kg	5/15	8/15	5/15
IAC 0.5 mg/Kg	2/15	4/15	2/15
IAC 2.5 mg/Kg	1/15	2/15	1/15
IAC 12.5 mg/Kg	2/15	7/15	2/15

Results





MEDESTEA
RESEARCH & PRODUCTION S.A.

Conclusions

1) The present experiment indicates that IAC (0.1 mg/Kg) shows a trend in reducing infarct volume at 72 hours after tMCAO in rats.
2) The results of the current studies indicate also that incidence of 0-lesions was mainly marked in 0.1 mg/Kg IAC treated group, in particular on the cortical infarct.
3) The present experiment may reflect neuroprotective properties of IAC after i.v. administration at 4, 6, 8 and 24 hours after tMCAO in rats.
4) The results show a positive trend in the sensory-motor behavior improvement when 0.1 mg/Kg of IAC was administrated i.v. at 4, 6, 8 and 24 hours after tMCAO in rats.

CEREBRICON

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A leader in the non-clinical screening of drug candidates against CNS disease targets.

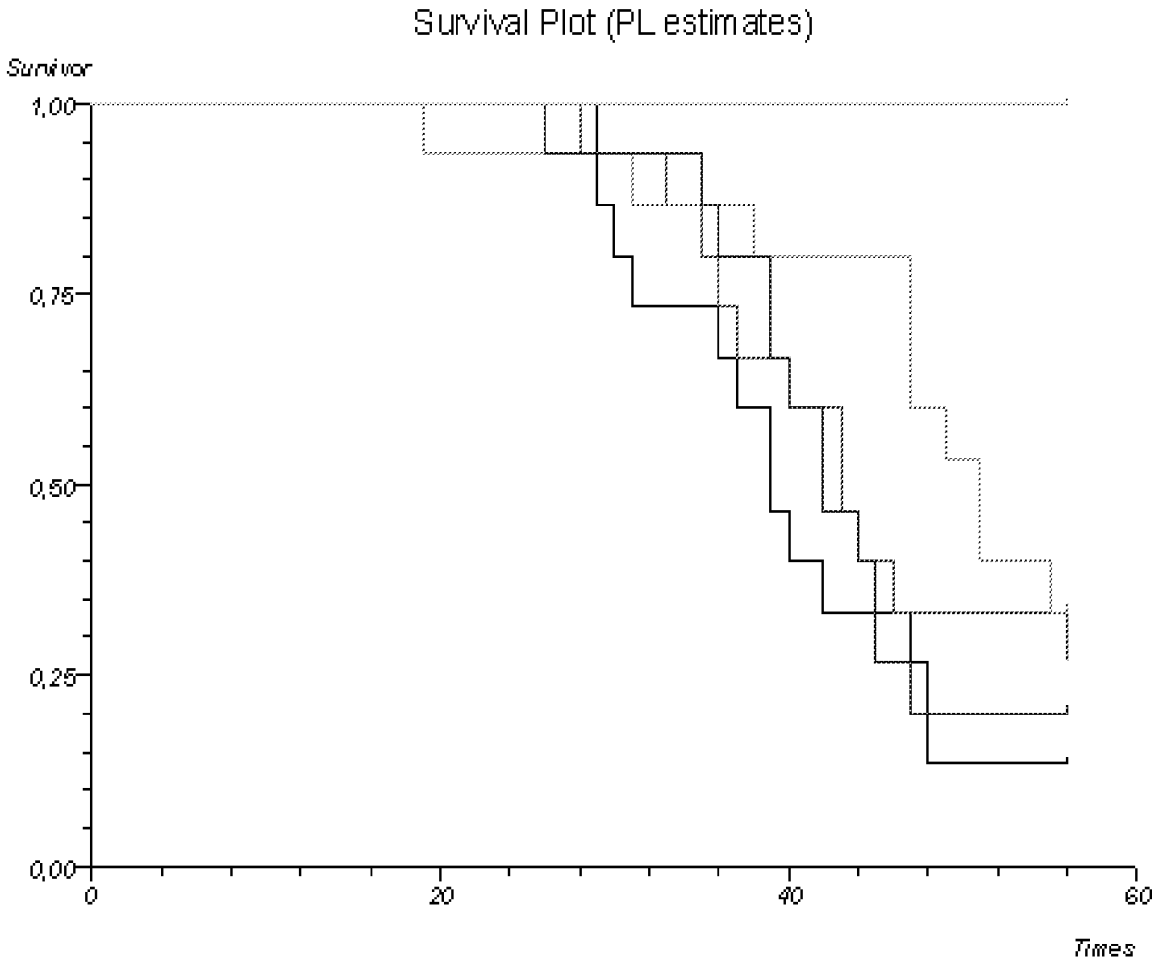
*Stroke prevention with **IAC** in Dahl Salt-Sensitive rats*

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Study outline

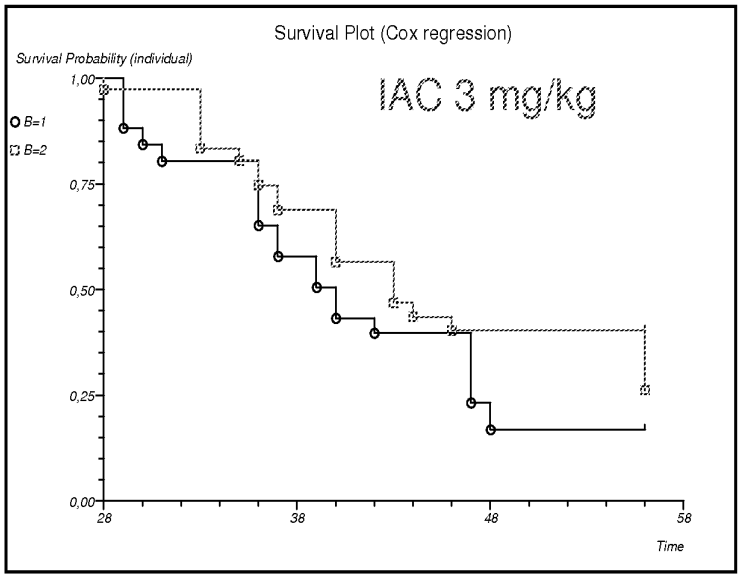
- Six-week-old male Dahl Salt-Sensitive rats (total N=70)
- 4 groups with High-Salt Diet treated from week 8 to week 11:
  - Veichle
  - IAC: 3, 10 and 30 mg/kg
- 1 group with normal-Salt Diet
- Evaluation of:
  - Body weight
  - T2- and T2*- MRI (once a week)
  - FOB and 28 NS (once a week)
  - Hemodynamics
  - Necropsy

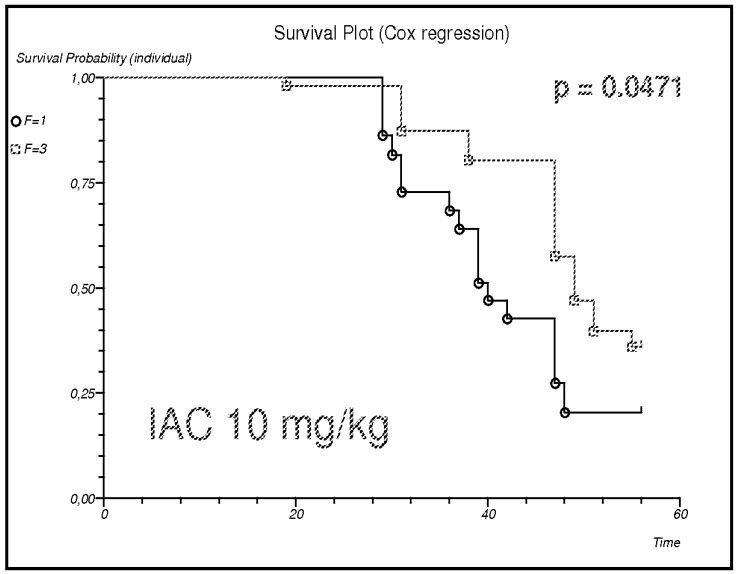
Results

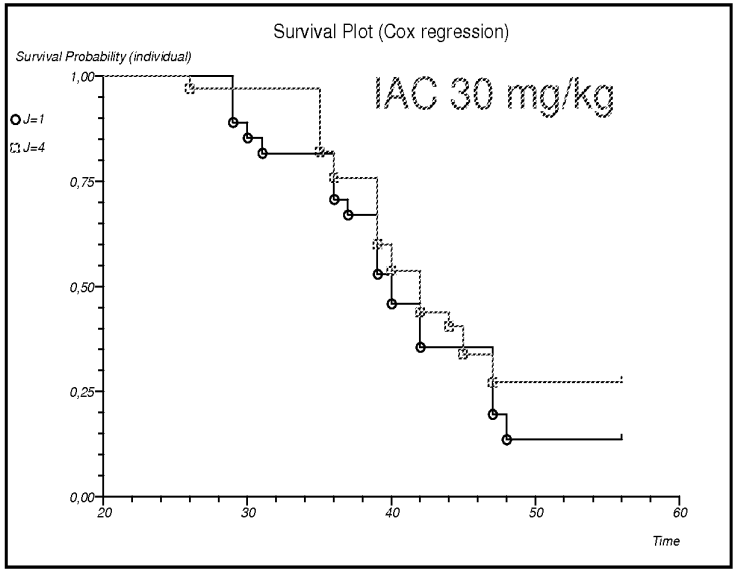


MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Results

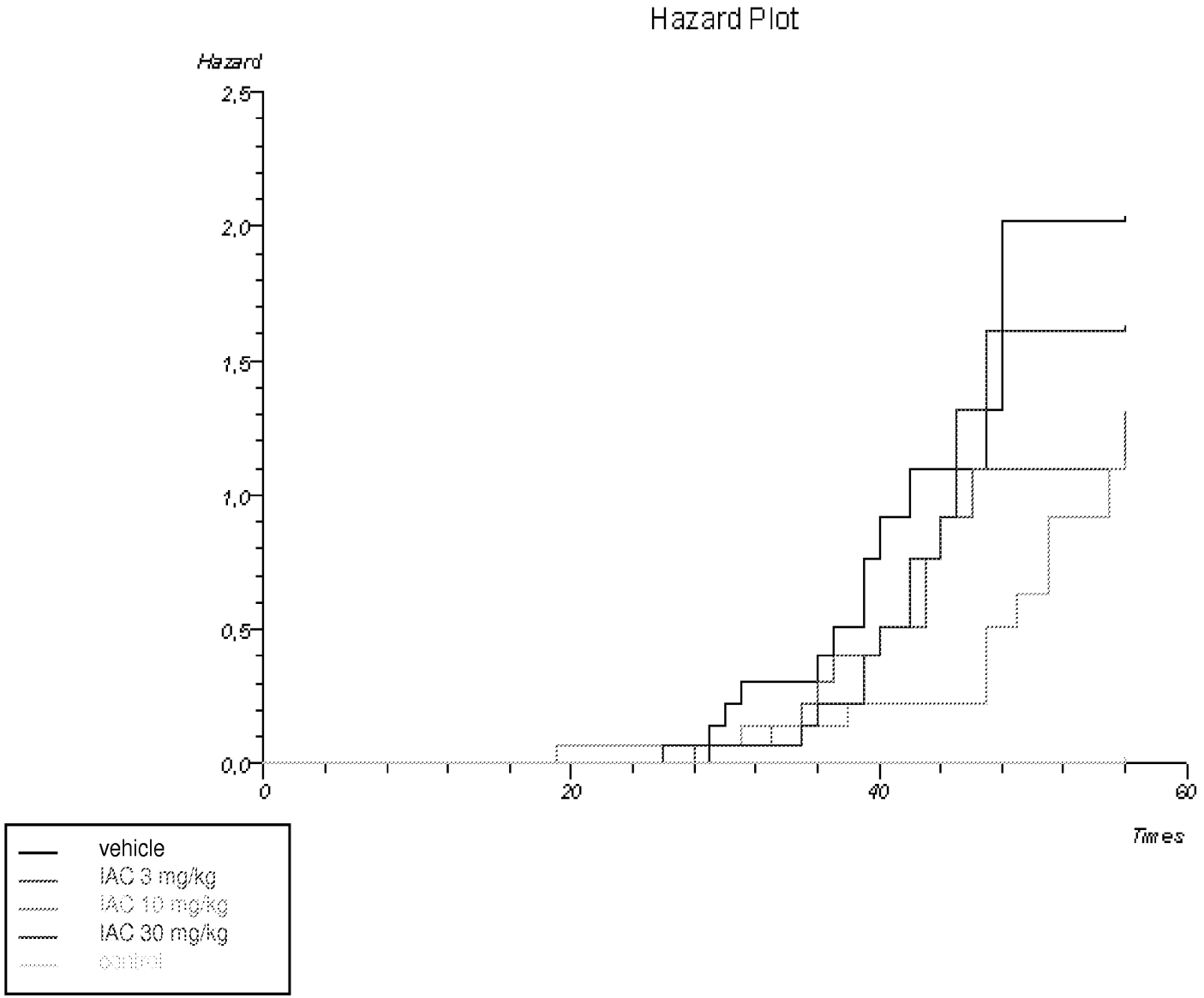




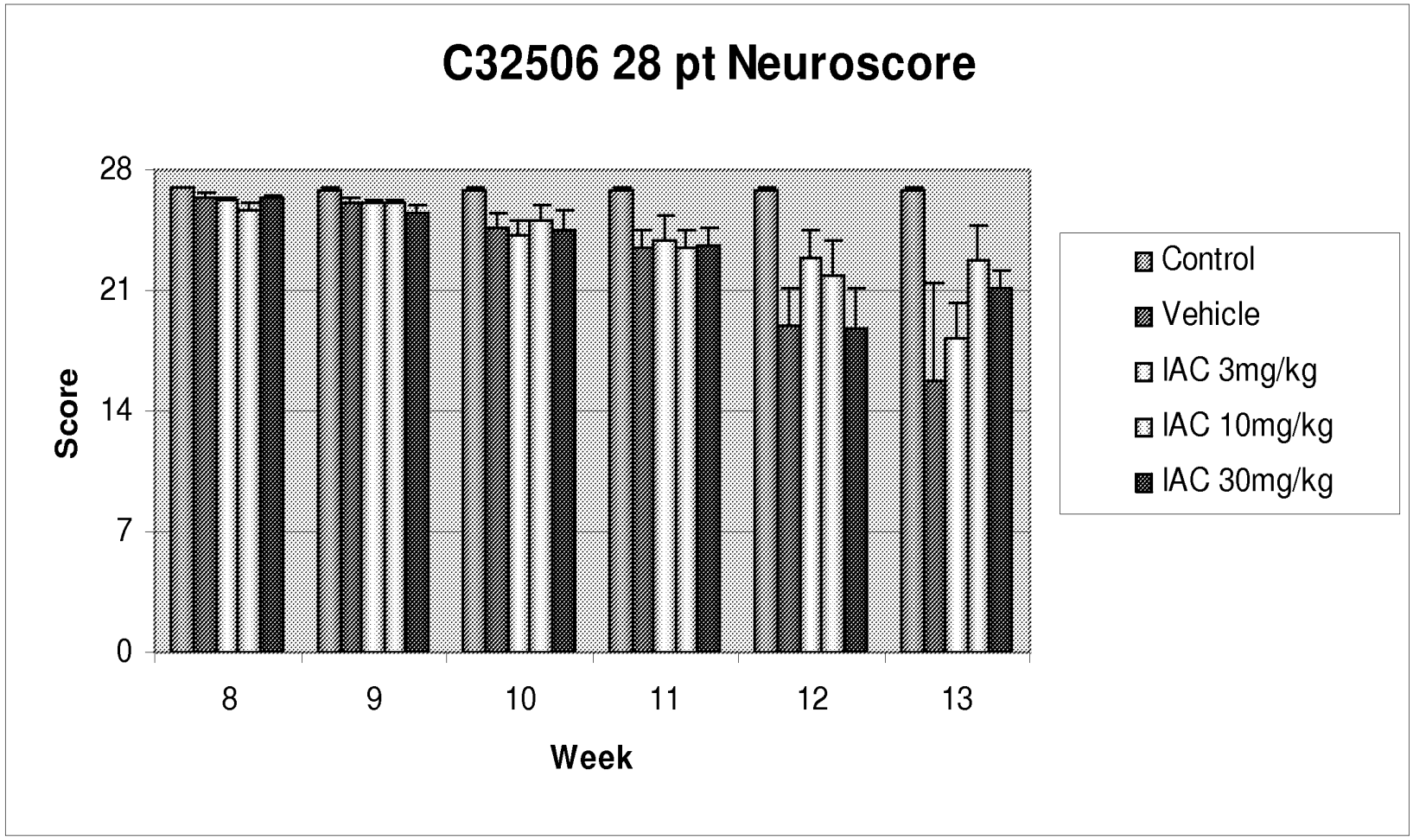


MEDESTEA
RESEARCH & PRODUCTION S.r.l.

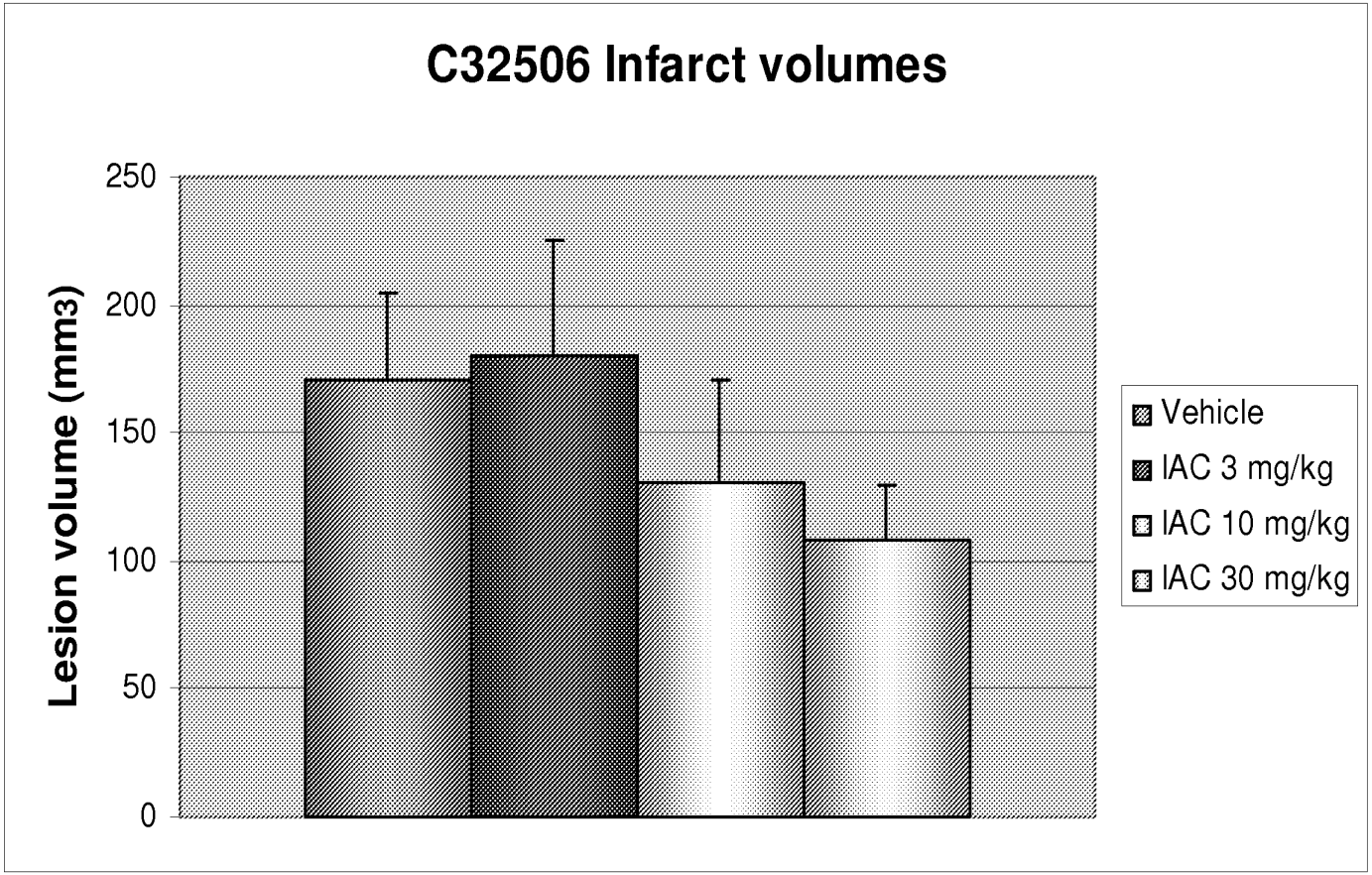
Results


Hazard Plot
Hazard
2,5
2,0
1,5
1,0
0,5
0,0
0
20
40
60
Times
vehicle
IAC 3 mg/kg
IAC 10 mg/kg
IAC 30 mg/kg
control

Results


C32506 28 pt Neuroscore
Score
28
21
14
7
0
8
9
10
11
12
13
Week
Control
Vehicle
IAC 3mg/kg
IAC 10mg/kg
IAC 30mg/kg

Results


C32506 Infarct volumes
Lesion volume (mm3)
250
200
150
100
50
0
Vehicle
IAC 3 mg/kg
IAC 10 mg/kg
IAC 30 mg/kg

Conclusion

1) Daily i.p. treatment with IAC (3, 10 or 30 mg/kg) did not significantly affect mortality when compared to vehicle group during 56 day follow-up period.

2) IAC 10 mg/kg treatment delayed mortality.

3) Sensory-motor functions (28-point NS test) were not significantly different between vehicle and IAC groups.

4) FOB test showed that proportions of normal findings in several test parameters were often higher in IAC groups when compared to vehicle group, especially on age weeks 8-10.

5) In vivo and ex vivo MRI result showed that the higher doses of IAC seem to decrease the infarct volume.

2006 - Atlanta, Georgia, Oct. 14-18

SOCIETY FOR NEUROSCIENCE

CEREBRICON

Neuroprotective Properties of IAC in tMCAO Model in Rats

J. Puoliväli[1], A. Nurmi[1], R. Pussinen[1], J. Yrjänheikki[1], A. Soleti[2], K. Bagate[3] and M. Paolini[4].

[1]Cerebricon Ltd, Kuopio, FINLAND, [2]Medestea Research, Torino, ITALY, [3]Forenap Pharma, Rouffach, FRANCE, [4]Dept. of Pharmacology, University of Bologna, Bologna, ITALY.

Background

Free radical scavenging and trapping agents exert neuroprotective properties in animal models of stroke. However, these compounds have limited therapeutic time window, unsatisfactory chemical/physical properties and are restricted to neutralize oxygen centered free radicals only. Here we investigated the effect of a novel, potent, broad range free radical neutralizer, IACVITA, on cerebral infarct volume, and sensory-motor and cognitive performance in transient Middle Cerebral Artery Occlusion model of stroke (tMCAO) in rats.

Materials & Methods

Animal groups and dosing. In first set of experiments, male Sprague-Dawley (250-350g) rats were subjected to 90 min tMCAO described by Koizumi et al. (1986) and treated with single i.p. administration of vehicle or 10, 30 or 90 mg/kg of IACVITA at 1, 3 or 6 h after the onset of tMCAO. Rats were monitored for 72 h after tMCAO. In a second set of experiments male Sprague-Dawley rats were subjected to 90 min tMCAO, and IACVITA 1 mg/kg was administered as an i.v. bolus at 1, 3 or 6 h after the onset of tMCAO and again at 24 h and 48 h after tMCAO. Rats were monitored for 22 days after tMCAO.

Behavioral testing. Sensory-motor performance was evaluated by 7- (modified from Zausinger et al. 2000) and 28 -point Neuroscore tests (NS) at 1, 24, 48, 72 h, 7d, 14d and 21 d after tMCAO. Cognitive testing was conducted using 1-day water maze protocol. On day 22 post-tMCAO, the rats were given a series of 5 trials, 1 hour apart in a large dark-colored tank (200 cm in diameter) filled with clear water at a temperature of 25.0±1.0°C. A 10 x 10-cm submerged platform (2 cm below water surface) was placed in the north-west (NW) quadrant of the pool. Each rat was given a maximum of 90 sec to find the submerged platform. This process was repeated a total of 5 times for each rat, each trial 1 hour apart. Open field test was performed on day 14 after tMCAO in an open-field arena (square 40×40×40 cm) and in which the number of vertical rearings was analyzed.

Infarct volume analysis. Cerebral infarct volume was evaluated with 2,3,5-triphenyl tetrazolium chloride (TTC) staining or with T2-weighed MRI at 72 h after tMCAO. TTC staining was performed on 2-mm sections and infarct volume was corrected for edema. T2-weighted MRI was performed with the use of a Varian Inova console interfaced to a 4.7 T horizontal magnet equipped with actively shielded gradient coils. A half-volume coil, driven in quadrature mode, was used for signal transmission and reception. For determination of infarct volume, T2-weighted multislice (12-14 continuous slices) images were acquired using double spin-echo sequence with adiabatic refocusing pulses TR = 3 s, TE = 80 ms, matrix size of 256x128, FOV of 35*35 mm², and a slice thickness of 1 mm.

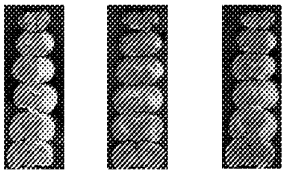
Results

Rats subjected to 90 min tMCAO presented a significantly lower neuroscore values in both 7- and 28 –point scale compared to pre-values in both sets of experiments. In i.p. administration studies rats treated with IACVITA 10 mg/kg at 1h after the onset of tMCAO had significantly higher 28 –point neuroscore at 72h when compared to vehicle group. In addition, rats treated with 90 mg/kg 6 h after the onset of tMCAO 28 –point neuroscore was higher at 24 h and 72 h after tMCAO when compared to vehicle group. In 7 –point scoring system, neuroscore was significantly higher in IACVITA 90 mg/kg 6 h group at 72 h after tMCAO when compared to vehicle group. In line with improvement in neuroscore values, infarct volumes were significantly lower in IACVITA 10 mg/kg 1 h and 90 mg/kg 6 h groups.

In i.v. administration studies, IACVITA 1 mg/kg administered 1 h after the onset of tMCAO improved 7 –point neuroscore values at 14 d, and 28 –point neuroscore values at 7 d after tMCAO when compared to vehicle group. T2-MRI revealed that infarct volumes were significantly smaller at 72 h when compared to vehicle group. In addition to neuroprotection and improved sensory-motor functions, IACVITA 1 mg/kg administered 1 h after the onset of tMCAO improved escape latency of the rats to the level of naive rats in water maze test. Moreover, in open field test number of vertical rearings was higher in rats treated with IACVITA 1 mg/kg 1 h after the onset of tMCAO than in vehicle treated rats.

Conclusions

This study demonstrates that IACVITA, a novel free radical neutralizer, has both acute and long term beneficial effects on sensory-motor and cognitive functions after focal cerebral ischemia in rats. In addition, IACVITA reduces lesion size as detected by MRI.



Vehicle | IACVITA 10 mg/kg i.p. 1h post MCAO | IACVITA 90 mg/kg i.p. 6h post MCAO

Figure 1. Representative coronal sections from rats subjected to 90 min tMCAO and treated with vehicle or IACVITA.

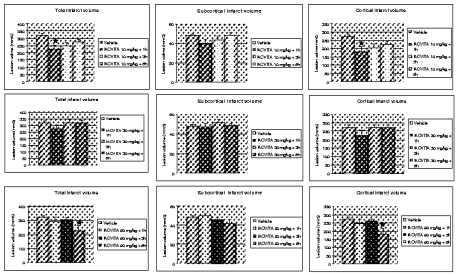


Figure 2. Total, cortical and subcortical infarct volumes from rats subjected to 90 min tMCAO, which were treated with vehicle or IACVITA 10, 30 or 90 mg/kg at 1, 3 or 6 h after the onset of tMCAO.

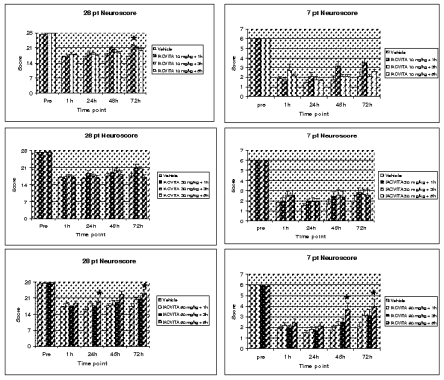
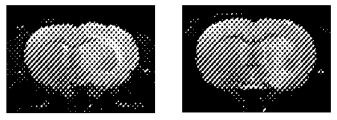


Figure 3. Seven (right panels) and 28 –point (left panels) neuroscore values from rats subjected to 90 min tMCAO, which were treated with vehicle or IACVITA 10, 30 or 90 mg/kg at 1, 3 or 6 h after the onset of tMCAO.



Vehicle | IACVITA 1 mg/kg i.v. 1h post MCAO

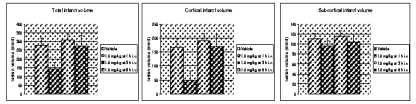


Figure 4. Representative T2-MRI images from rats subjected to 90 min tMCAO and treated with vehicle or IACVITA (upper left and right panel). Average total, cortical and subcortical infarct volumes were significantly lower in rats treated with IACVITA 1 mg/kg 1 h after the onset of tMCAO.

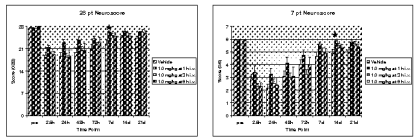


Figure 5. Seven (right panel) and 28 –point (left panel) neuroscore values from rats subjected to 90 min tMCAO and treated with vehicle or IACVITA 1 mg/kg at 1, 3 or 6 h after the onset of tMCAO. IACVITA administered 1 h after the onset of tMCAO significantly improved 7 and 28 –point neuroscore values on day 7 and 14, respectively.

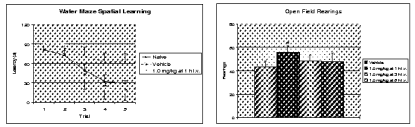


Figure 6. Escape latency in water maze test on day 22 and the number of rearings in open field test on day 14 after tMCAO. IACVITA administered 1 h after the onset of tMCAO significantly improved the performance in both tests.

2007 - Liverpool, UK, Apr. 1-4

CEREBRICON

Neuroprotective Properties of IAC in tMCAO Model in Rats

Antti Nurmi[1], Jukka Puoliväli[1], Raimo Pussinen[1], Antonio Soleti[2], Karim Bagate[3], Moreno Paolini[4], Robert I. Grundy[1], Juha Yrjänheikki[1]

[1]Cerebricon Ltd, Kuopio, FINLAND, [2]Medestea Research, Torino, ITALY, [3]Forenap Pharma, Rouffach, FRANCE, [4]Dept. of Pharmacology, University of Bologna, Bologna, ITALY.

Background

Free radical scavenging and trapping agents exert neuroprotective properties in animal models of stroke. However, these compounds have limited therapeutic time window, unsatisfactory chemical/physical properties and are restricted to neutralize oxygen centered free radicals only. Here we investigated the effect of a novel, potent, broad range free radical neutralizer, IAC, on cerebral infarct volume, and sensory-motor and cognitive performance in transient Middle Cerebral Artery Occlusion model of stroke (tMCAO) in rats.

Materials & Methods

- Male Sprague-Dawley (250-350g) rats were subjected to 90 min tMCAO described by Koizumi et al. (1986).
- **Expt 1:** Animals treated with single **i.p.** administration of vehicle or 10, 30 or 90 mg/kg of IAC at 1, 3 or 6 h after the onset of tMCAO.
- Rats were monitored for 72 h after tMCAO.
- **Expt 2:** IAC 1 mg/kg was administered as an **i.v**. bolus at 1, 3 or 6 h after the onset of tMCAO and again at 24 h and 48 h after tMCAO. Rats were monitored for 22 days after tMCAO.
- In all experiments sensory-motor performance was evaluated by 7- (modified from Zausinger et al. 2000) and 28 -point Neuroscore tests (NS) at 1, 24, 48, 72 h, 7d, 14d and 21 d after tMCAO.
- Cerebral infarct volume was evaluated with TTC staining or with T2-weighed MRI at 72 h after tMCAO.
- TTC staining was performed on 2-mm sections and infarct volume was corrected for edema.
- T2-weighted MRI was performed with the use of a Varian Inova console interfaced to a 4.7 T horizontal magnet equipped with actively shielded gradient coils. A half-volume coil, driven in quadrature mode, was used for signal transmission and reception. For determination of infarct volume, T2-weighted multislice (12-14 continuous slices) images were acquired using double spin-echo sequence with adiabatic refocusing pulses TR = 3 s, TE = 80 ms, matrix size of 256x128, FOV of 35*35 mm^2, and a slice thickness of 1 mm.

Results

Expt 1: i.p. Administration

- Rats treated with IAC 10 mg/kg at 1h after the onset of tMCAO had significantly higher 28 –point neuroscore at 72h when compared to vehicle group.
- Rats treated with 90 mg/kg 6 h after the onset of tMCAO 28 –point neuroscore was higher at 24 h and 72 h after tMCAO when compared to vehicle group. In 7 –point scoring system, neuroscore was significantly higher in IAC 90 mg/kg 6 h group at 72 h after tMCAO when compared to vehicle group.
- In line with improvement in neuroscore values, infarct volumes were significantly lower in IAC 10 mg/kg 1 h and 90 mg/kg 6 h groups.

Expt 2: i.v. Administration

- IAC 1 mg/kg administered 1 h after the onset of tMCAO improved 7 –point neuroscore values at 14 d, and 28 –point neuroscore values at 7 d after tMCAO when compared to vehicle group.
- T2-MRI revealed that infarct volumes were significantly smaller at 72 h when compared to vehicle group.

Conclusions

This study demonstrates that IAC, a novel free radical neutralizer, has both acute and long term beneficial effects on sensory-motor functions after focal cerebral ischemia in rats. In addition, IAC reduces lesion size as detected by histology and MRI. These data indicate the potential of IAC as a therapeutic for ischeamic stroke.

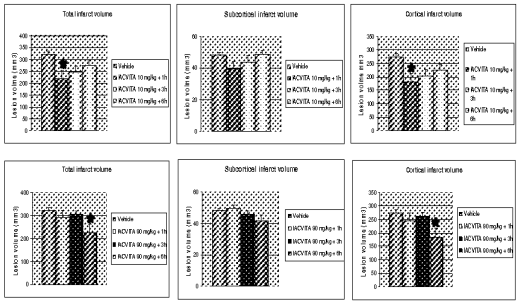


Figure 1. Total, cortical and subcortical infarct volumes from rats subjected to 90 min tMCAO, which were treated with vehicle or IAC 10 or 90 mg/kg at 1, 3 or 6 h after the onset of tMCAO. (*p<0.05, ANOVA)

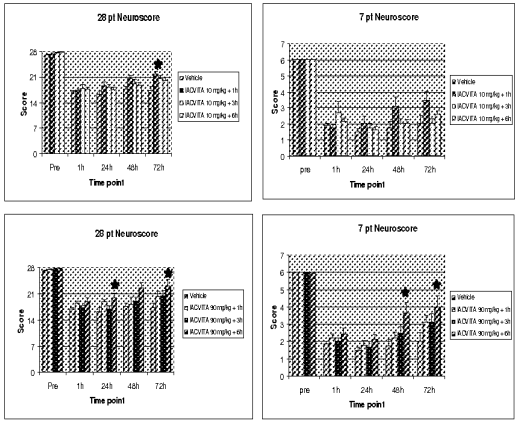
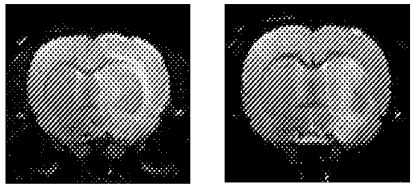


Figure 2. Seven (right panels) and 28 –point (left panels) neuroscore values from rats subjected to 90 min tMCAO, which were treated with vehicle or IAC 10, 30 or 90 mg/kg at 1, 3 or 6 h after the onset of tMCAO. (*p<0.05, ANOVA)



Vehicle

IAC 1 mg/kg i.v.
1h post MCAO

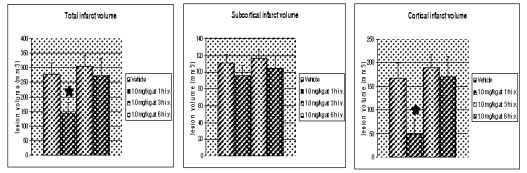


Figure 3. Representative T2-MRI images from rats subjected to 90 min tMCAO and treated with vehicle or IAC (upper left and right panel). Average total and cortical infarct volumes were significantly lower in rats treated with IAC 1 mg/kg 1 h after the onset of tMCAO. (*p<0.05, ANOVA)

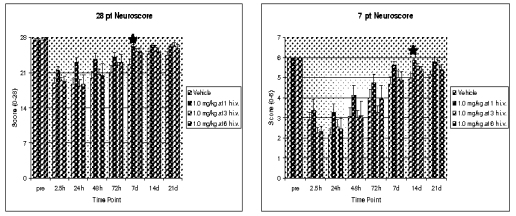


Figure 4. Seven (right panel) and 28 –point (left panel) neuroscore values from rats subjected to 90 min tMCAO and treated with vehicle or IAC 1 mg/kg at 1, 3 or 6 h after the onset of tMCAO. IAC administered 1 h after the onset of tMCAO significantly improved 7 and 28 –point neuroscore values on day 7 and 14, respectively. (*p<0.05, ANOVA)

IAC diabetes

EXHIBIT 3

University of Pisa

Department of Endocrinology and Metabolism.

The in vivo and in vitro IAC activity on diabetes models.

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

IN VITRO IAC STUDY

Langherans islets isolated from no-diabetic subjects.

Insulin static secretion (μU/ml)	
	I.S.
Ctrl	2.03±0.4
Glucose 22.2 mM	1.13±0.2
Glucose + IAC 1 μM	1.44±0.5
Glucose + IAC 10 μM	1.78±0.5*
Glucose + IAC 100 μM	1.50±0.4

*p < 0.01

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Langherans islets isolated from T2 diabetic subjects.

Insulin static secretion (µU/ml)	
	I.S.
Healthy subjects	**2.03±0.4**
T2 D	**0.87±0.2**
T2 D + IAC 1 µM	**1.13±0.3**
T2D + IAC 10 µM	**1.55±0.4**
T2 D + IAC 100 µM	**1.65±0.5**

T2 D = Type 2 diabetes

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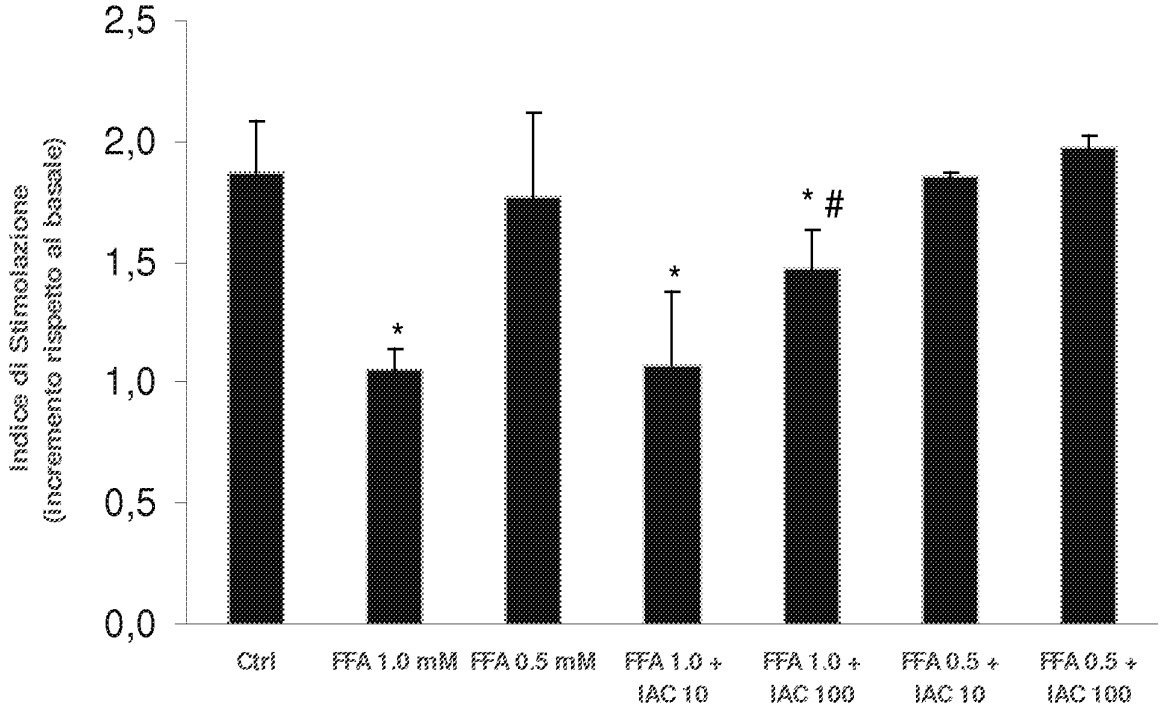
Langherans islets isolated from no-diabetic subjects. 24 hours-induction with Free Fatty Acid (FFA).

Beta-cells activity is expressed by the insulin levels after stimulation with high doses of glucose

	IS
CTRL	1.9±0.2
FFA 0.5 mM	1.8±0.3
FFA 1.0 mM	1.0±0.09*
FFA 0.5 mM + IAC 10 µM	1.8±0.02
FFA 0.5 mM + IAC 100 µM	2.0±0.05
FFA 1.0 mM + IAC 10 µM	1.1±0.3*
FFA 1.0 mM + IAC 100 µM	1.5±0.2*#



*=p<0.05 vs Ctrl; #=p<0.05 vs FFA 1.0 mM; Bonferroni test

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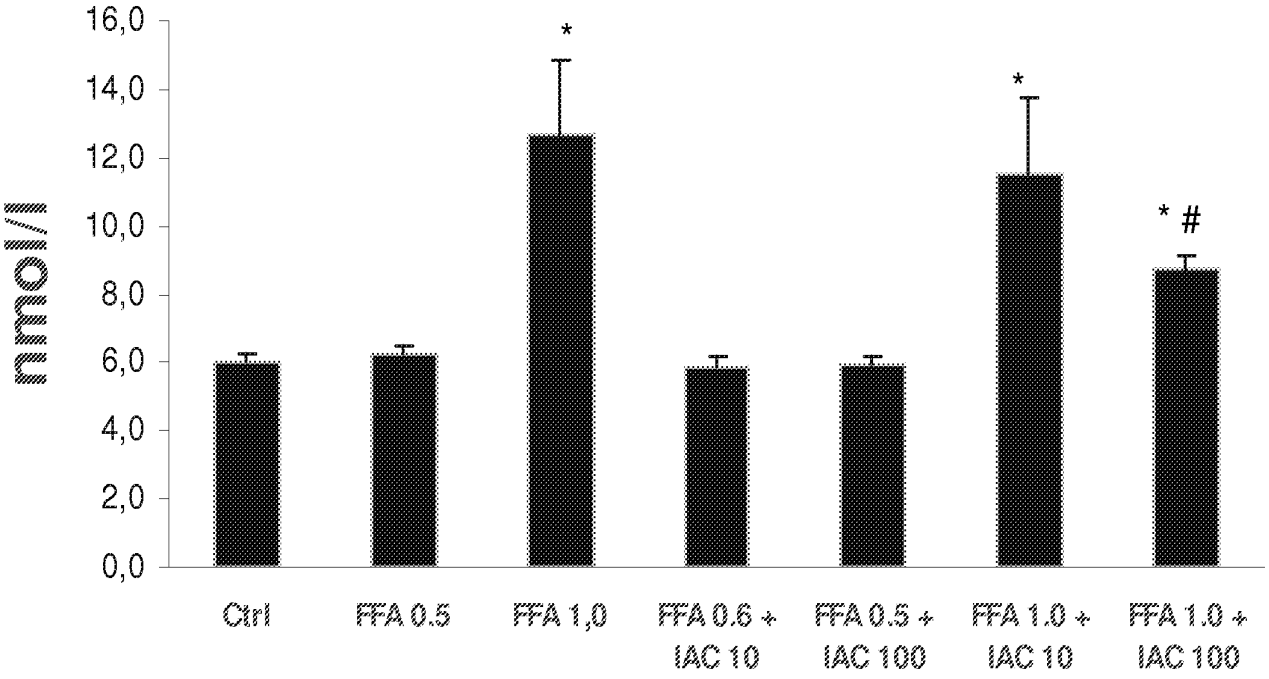
Langherans islets isolated from no-diabetic subjects.

24 hours Free Fatty Acid (FFA) induction.

Evaluation of Oxidative stress: nitrotyrosine

	nmol/l
CTRL	6,00±0.26
FFA 0.5 mM	6,23±0.23
FFA 1.0 mM	12,7±2.15*
FFA 0.5 mM + IAC 10 µM	5,84±0.33
FFA 0.5 mM + IAC 100 µM	5,92±0.27
FFA 1.0 mM + IAC 10 µM	11,55±2.18*
FFA 1.0 mM + IAC 100 µM	8,76±0.37*#



*=p<0.05 vs Ctrl; #=p<0.05 vs FFA 1.0 mM; Bonferroni test

MEDESTEA
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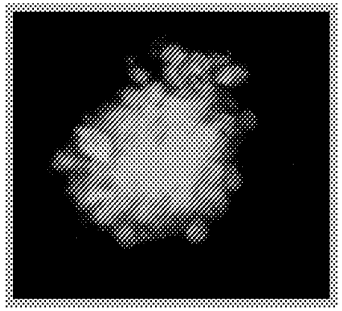
Langherans islets isolated from no-diabetic subjects.

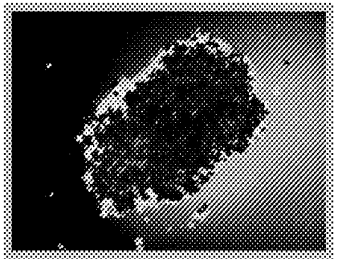
24 hours Free Fatty Acid (FFA) induction.

CTRL

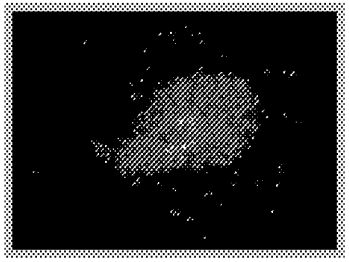


The Green fluorescence indicates vital cells, the red fluorescence indicate the not-vital cells

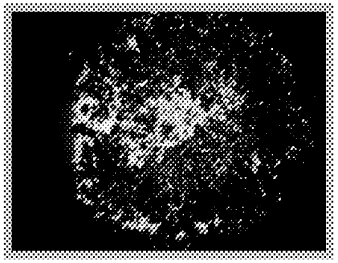
FFA 0.5 mM



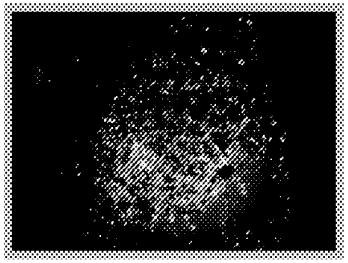
FFA 1.0 mM



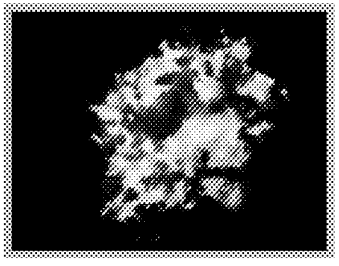
FFA 0.5 mM
+ IAC 10 µM



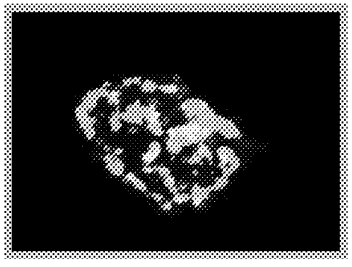
FFA 1.0 mM
+ IAC 10 µM



FFA 0.5 mM
+ IAC 100 µM



FFA 1.0 mM
+ IAC 100 µM



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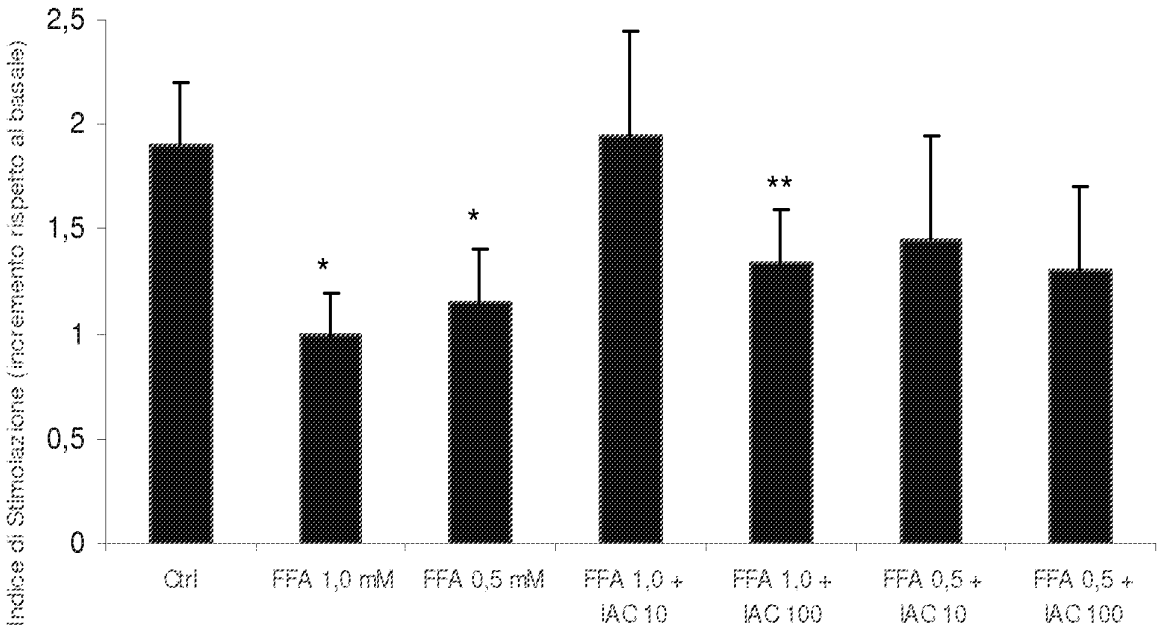
Langherans islets isolated from no-diabetic subjects. 48 hours-induction with Free Fatty Acid (FFA).

Beta-cells activity is expressed by the insulin levels after stimulation with high doses of glucose

	IS
CTRL	1.9±0.3
FFA 0.5 mM	1.15±0.25*
FFA 1.0 mM	1.0±0.2*
FFA 0.5 mM + IAC 10 µM	1.45±0.5
FFA 0.5 mM + IAC 100 µM	1.3±0.4
FFA 1.0 mM + IAC 10 µM	1.95±0.5
FFA 1.0 mM + IAC 100 µM	1.34±0.25**



*=p<0.01 vs Ctrl; **=p<0.05 vs Ctrl; test-t

MEDESTEA
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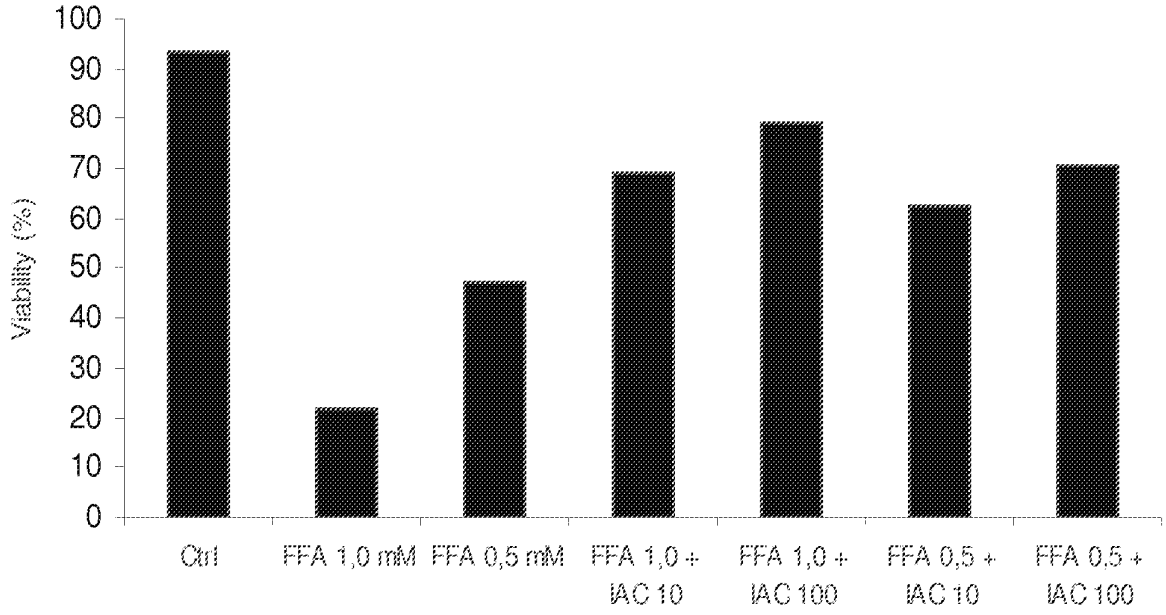
Langherans islets isolated from no-diabetic subjects. 48 hours-induction with Free Fatty Acid (FFA).

Viability cells

	%
CTRL	93.2
FFA 0.5 mM	47.1
FFA 1.0 mM	22
FFA 0.5 mM + IAC 10 µM	62.3
FFA 0.5 mM + IAC 100 µM	70.5
FFA 1.0 mM + IAC 10 µM	69.2
FFA 1.0 mM + IAC 100 µM	79.1



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CONCLUSIONS

Type-2 diabetic islets, for which an abnormal oxidative stress status was observed, were able to achieve a normalization of gene expression, glucose-stimulated insulin secretion and nitrotyrosine levels when pre-exposed for 24 h to IACVITA.

These results support the concept that oxidative stress plays a role in type-2 diabetes beta-cell dysfunction; therapy with IACVITA could therefore be an interesting adjunctive pharmacological approach to the treatment of type-2 diabetes.

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Published in 2007 on Diabetes & Metabolism

Insulin secretion defects of human type 2 diabetic... [Diabetes Metab. 2007] - PubMed Result - Microsoft Internet Explorer

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1: Diabetes Metab. 2007 Nov;33(5):340-5. Epub 2007 Jul 9.

Full text on Masson.it Subscription required. Links

Insulin secretion defects of human type 2 diabetic islets are corrected in vitro by a new reactive oxygen species scavenger.

Lupi R, Del Guerra S, Mancarella R, Novelli M, Valgimigli L, Pedulli GF, Paolini M, Soleti A, Filipponi F, Mosca F, Boggi U, Del Prato S, Masiello P, Marchetti P.

Dipartimento di Endocrinologia e Metabolismo, University of Pisa, Italy.

Oxidative stress is a putative mechanism leading to beta-cell damage in type 2 diabetes. We studied isolated human pancreatic islets from type 2 diabetic and non-diabetic subjects, matched for age and body mass index. Evidence of increased oxidative stress in diabetic islets was demonstrated by measuring nitrotyrosine concentration and by electron paramagnetic resonance. This was accompanied by reduced glucose-stimulated insulin secretion, as compared to non-diabetic islets (Stimulation Index, SI: 0.9 +/- 0.2 vs. 2.0 +/- 0.4, P<0.01), and by altered expression of insulin (approximately -60%), catalase (approximately +90%) and glutathione peroxidase (approximately +140%). When type 2 diabetic islets were pre-exposed for 24 h to the new antioxidant bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)decandioate di-hydrochloride, nitrotyrosine levels, glucose-stimulated insulin secretion (SI: 1.6+/-0.5) and gene expressions improved/normalized. These results support the concept that oxidative stress may play a role in type 2 diabetes beta-cell dysfunction; furthermore, it is proposed that therapy with antioxidants could be an interesting adjunctive pharmacological approach to the treatment of type 2 diabetes.

PMID: 17616474 [PubMed - in process]

Related Links

- Functional and molecular defects of pancreatic islets in human type 2 diabetes. [Diabetes. 2005]
- Role of oxidative stress in pancreatic beta-cell dysfunction [Ann N Y Acad Sci. 2004]
- Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis [J Clin Endocrinol Metab. 2004]
- Probucol preserves pancreatic beta-cell function through reduction of oxidative stress in t [Diabetes Res Clin Pract. 2002]
- Evolution of beta-cell dysfunction in the male Zucker diabetic fatty rat. [Diabetes. 1995]

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Internet

IN VIVO IAC STUDY

- Niddm mice model
- Diabetes inducted with STZ + NA
- 5 groups (8 mice/group)
  - Control
  - STZ-NA
  - STZ-NA + IAC 15 mg/Kg
  - STZ-NA + IAC 30 mg/Kg
  - STZ-NA + IAC 60 mg/Kg

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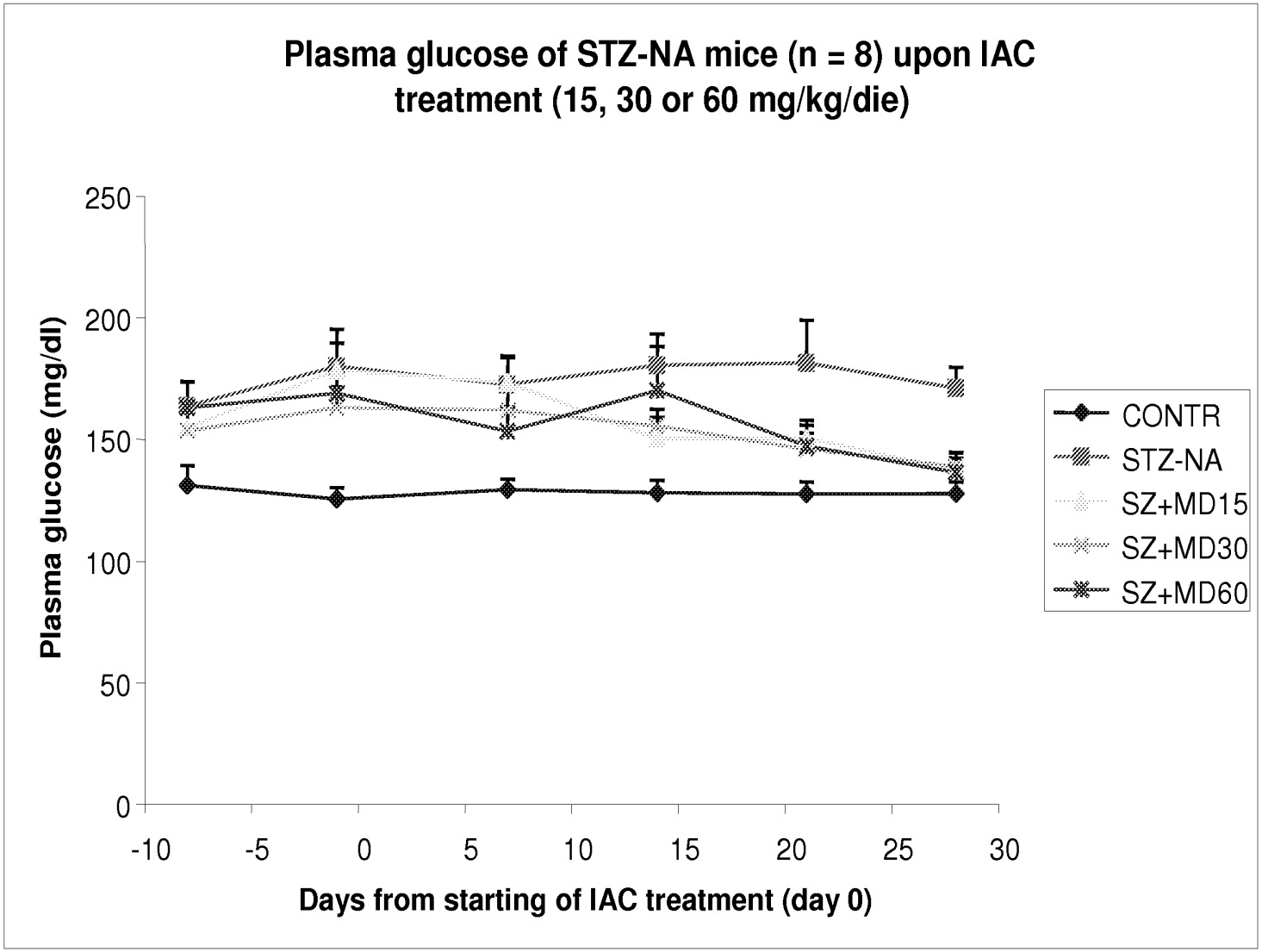
IAC activity in a Niddm Mice model.



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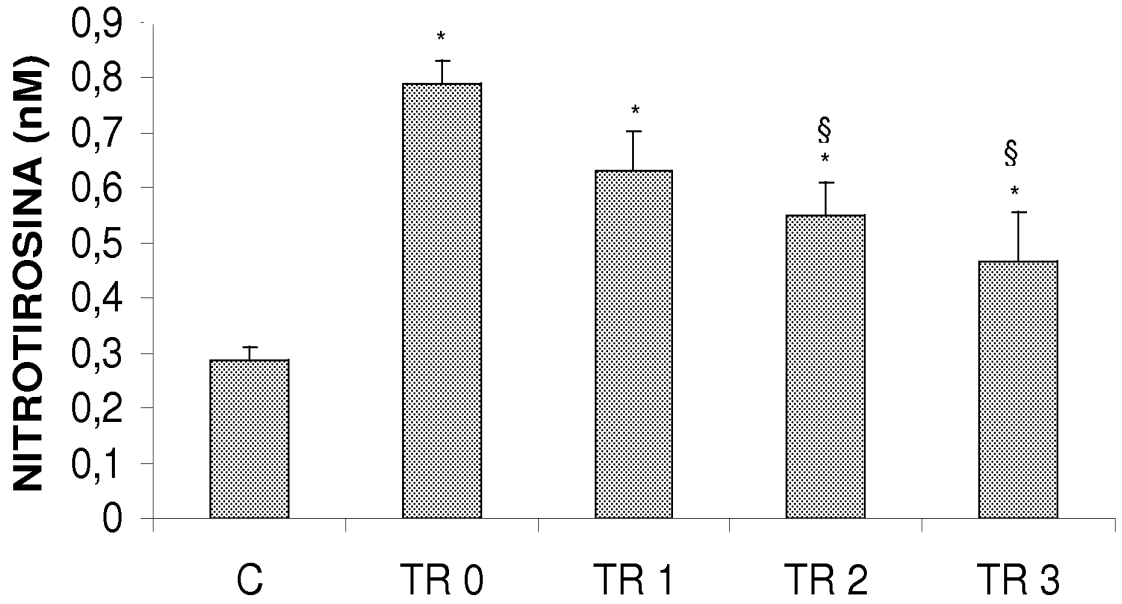
Plasma Nitrotyrosine contents in STZ-NA treated mice.



* $p < 0.01$ vs. C § $p < 0.01$ vs. TR 0

C = control; TR 0 = STZ-NA; TR 1 = STZ-NA + IAC 60 mg/kg;
TR 2 = STZ-NA + IAC 30 mg/kg; TR 3 = STZ-NA + IAC 15 mg/kg

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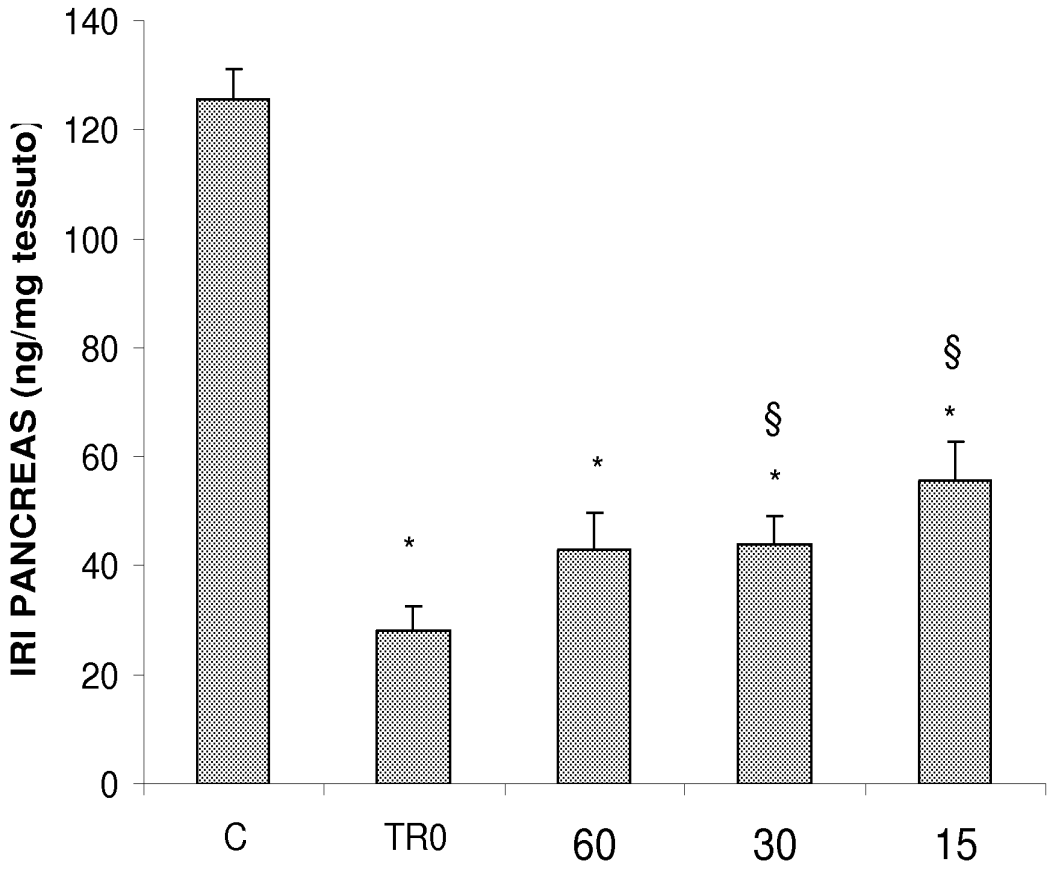
Pancreatic insulin contents in STZ-NA treated mice.



* p < 0.01 vs. C § p < 0.01 vs. TR 0

C = control; TR 0 = STZ-NA; TR 1 = STZ-NA + IAC 60 mg/kg;
TR 2 = STZ-NA + IAC 30 mg/kg; TR 3 = STZ-NA + IAC 15 mg/kg

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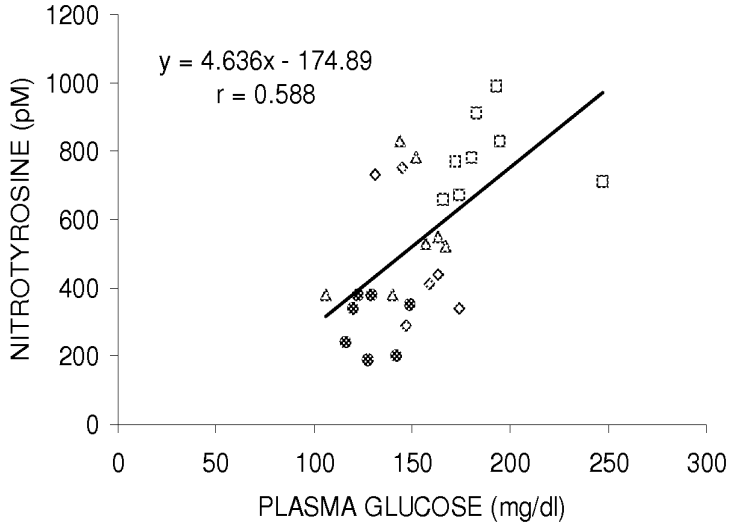
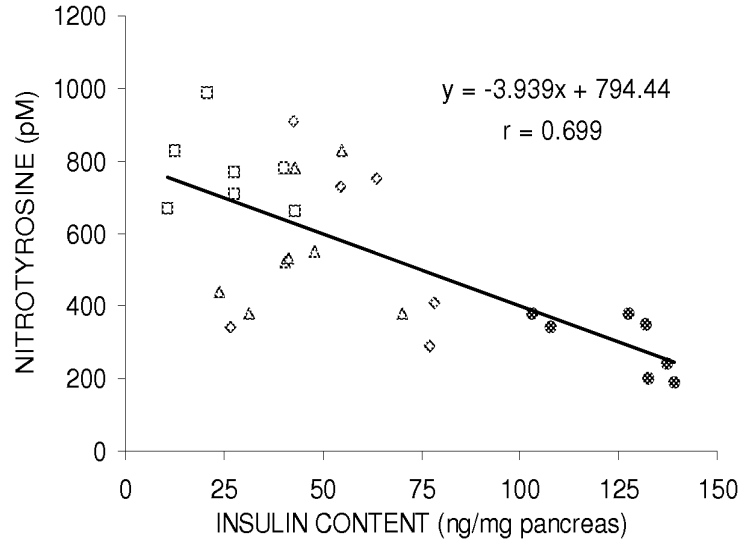
Correlation between glycaemic values or pancreatic insulin content and plasma nitrotyrosine levels.





IN VIVO IAC STUDY

- Niddm mice model
- Diabetes inducted with STZ + NA
- 5 groups (8 mice/group)
  - Control
  - STZ-NA
  - STZ-NA + IAC 7.5 mg/Kg
  - STZ-NA + IAC 15 mg/Kg
  - STZ-NA + IAC 30 mg/Kg

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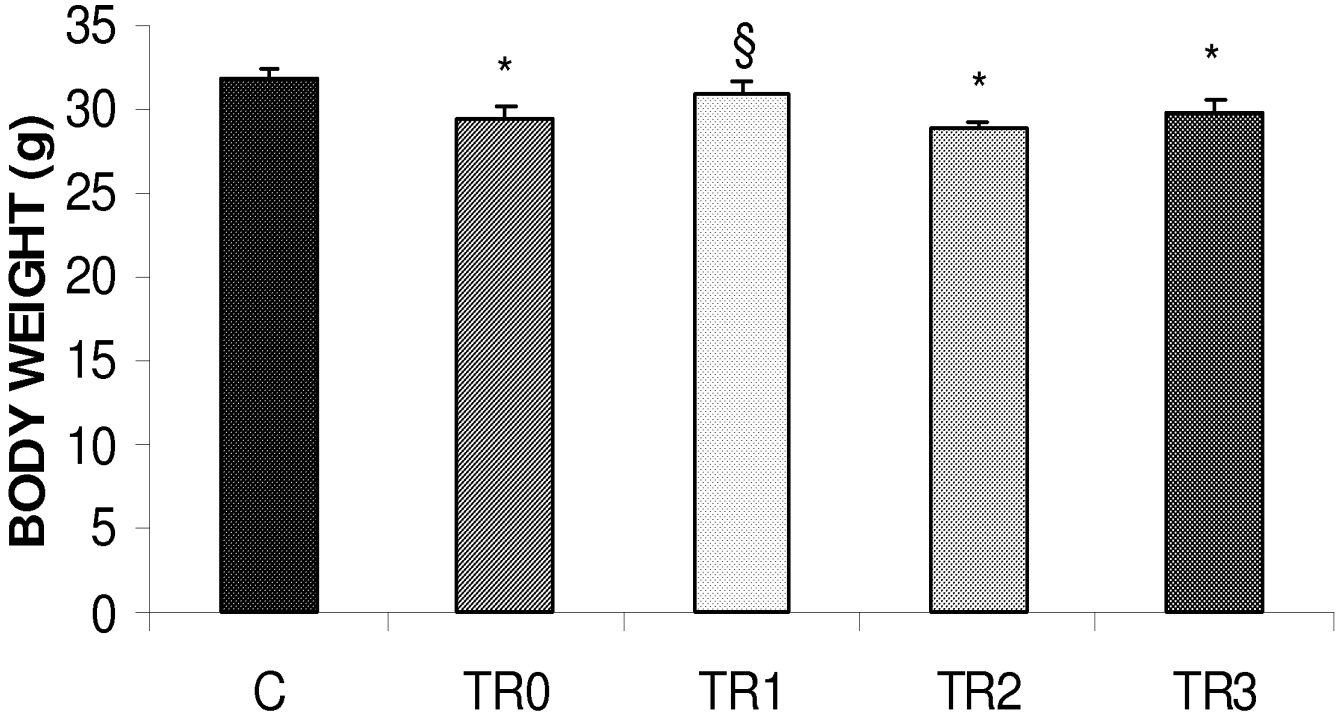
BODY WEIGHT



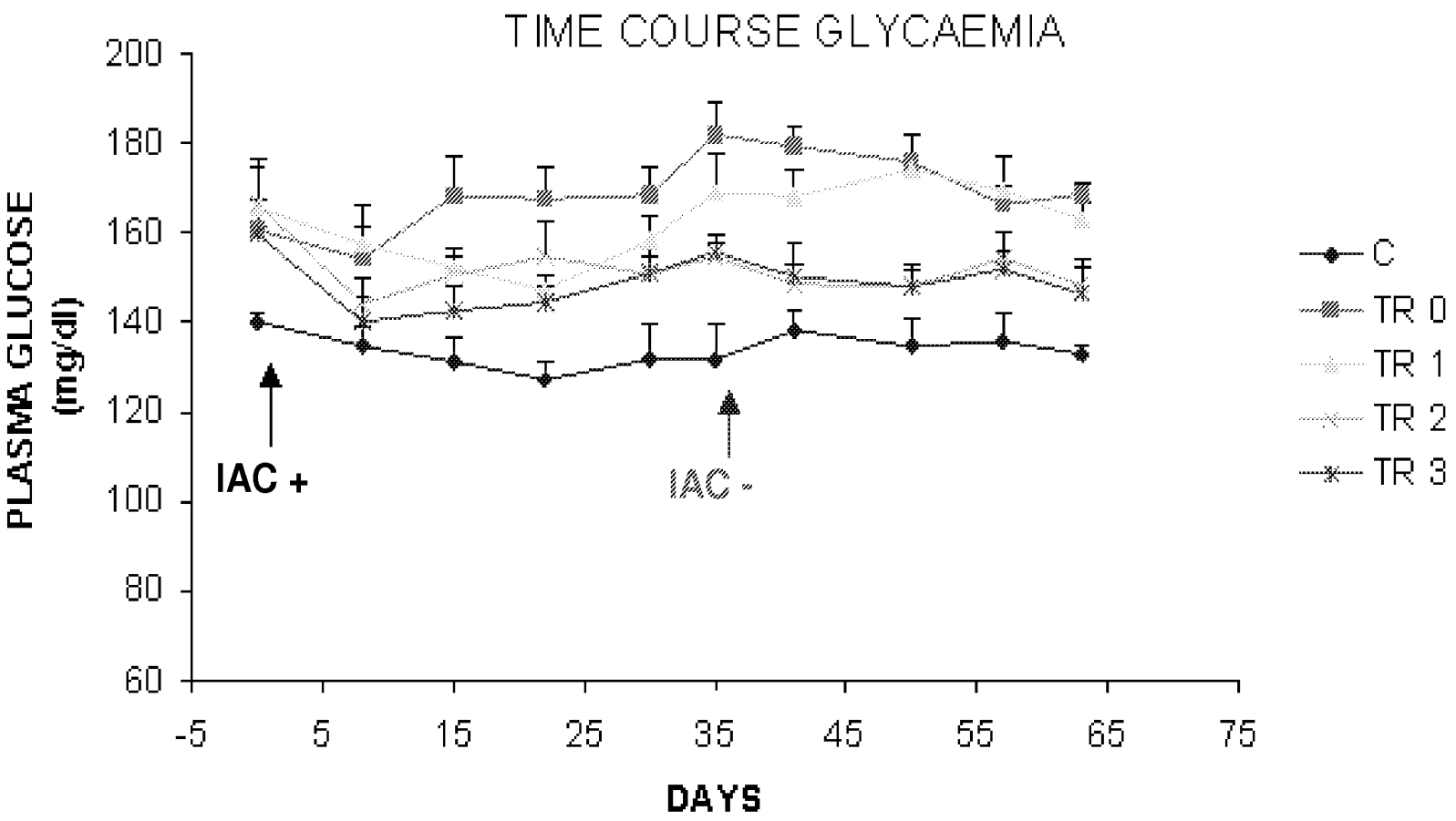
* p<0.05 at least vs. CONTR

§ p<0.05 vs. SZ-MD30

C = Control
TR0 = STZ-NA
TR1 = STZ-NA + IAC 7,5 mg/Kg
TR2 = STZ-NA + IAC 15 mg/Kg
TR3 = STZ-NA + IAC 30 mg/Kg

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

PLASMA GLUCOSE LEVELS



C = Control
TR0 = STZ-NA
TR1 = STZ-NA + IAC 7,5 mg/Kg
TR2 = STZ-NA + IAC 15 mg/Kg
TR3 = STZ-NA + IAC 30 mg/Kg

MEDESTEA
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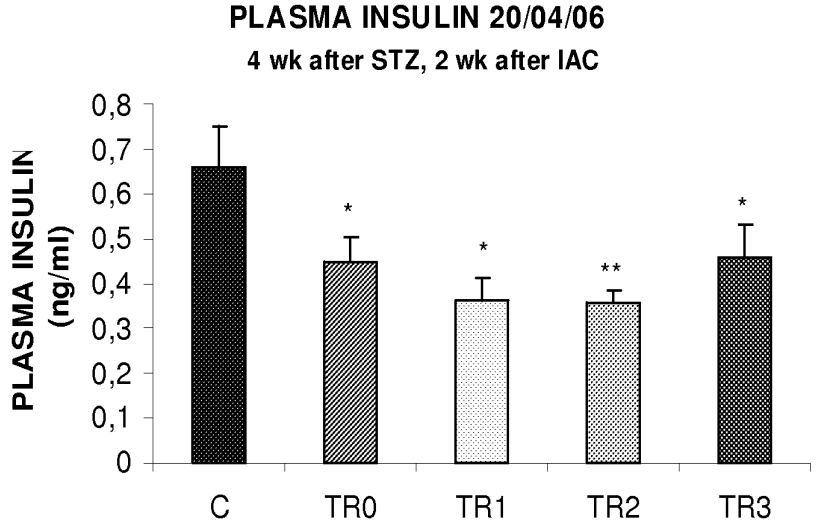
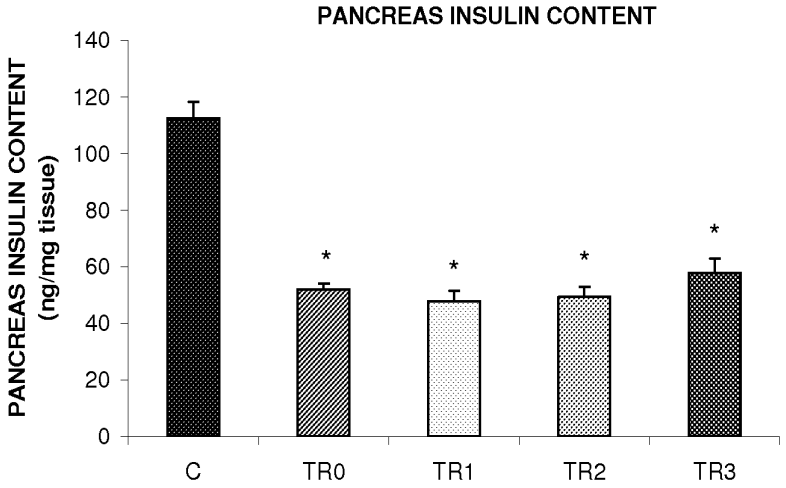
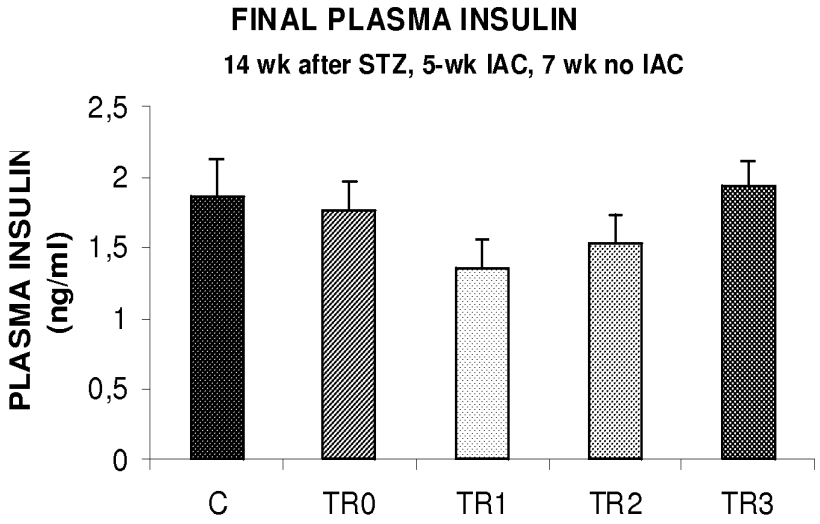
PLASMA AND PANCREATIC INSULIN LEVELS







* p<0.05 vs. CONTR

** p<0.01 vs.CONTR

C = Control
TR0 = STZ-NA
TR1 = STZ-NA + IAC 7,5 mg/Kg
TR2 = STZ-NA + IAC 15 mg/Kg
TR3 = STZ-NA + IAC 30 mg/Kg

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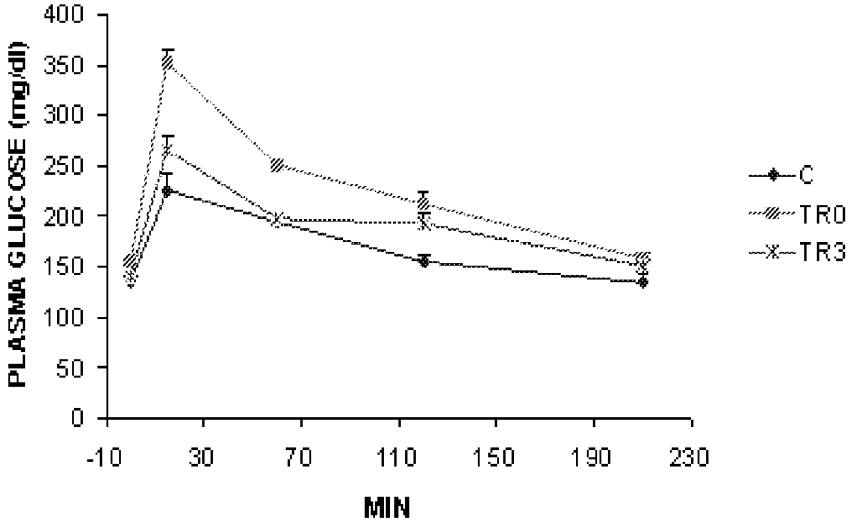
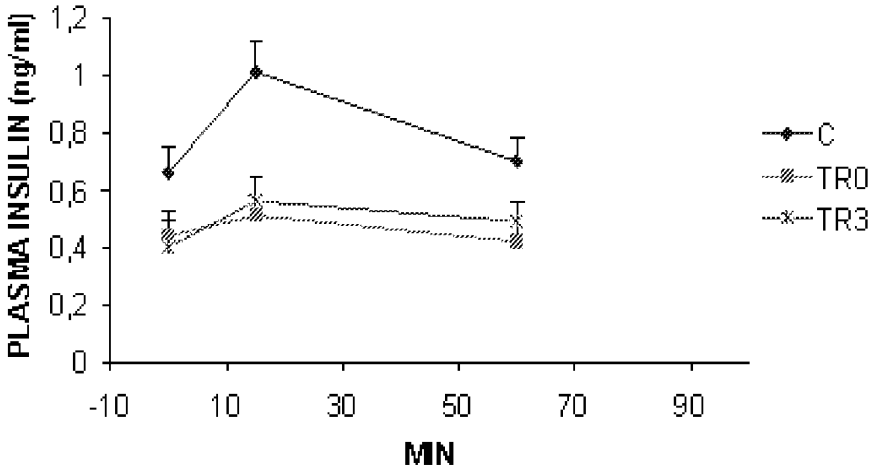
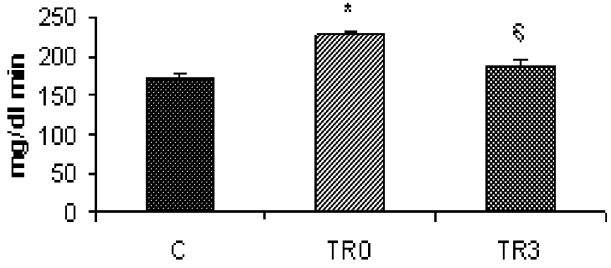
PLASMA GLUCOSE and PLASMA INSULIN LEVELS after GTT

4 wk after STZ / 2 wk IAC

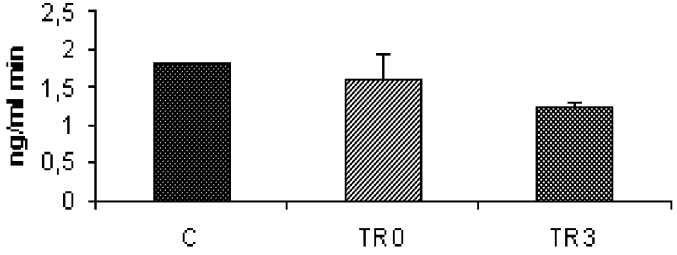




AUC



AUC



C = Control
TR0 = STZ-NA
TR3 = STZ-NA + IAC 30 mg/Kg

* p<0.05 vs. CONTR § p<0.05 vs. STZ-NA

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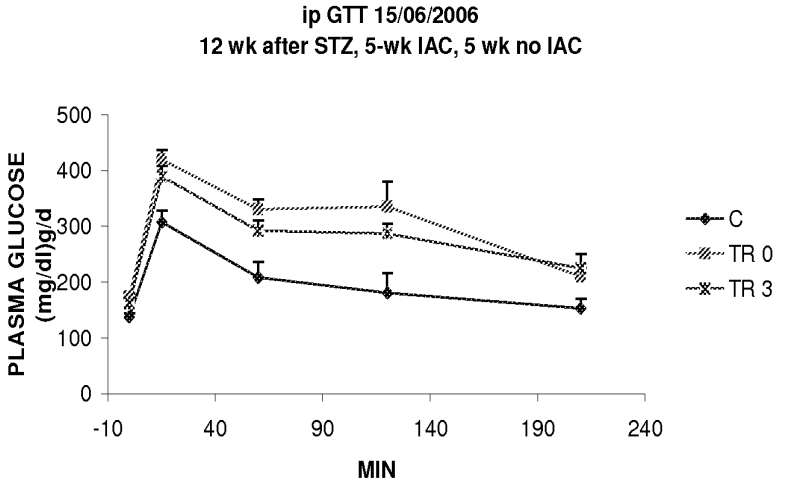
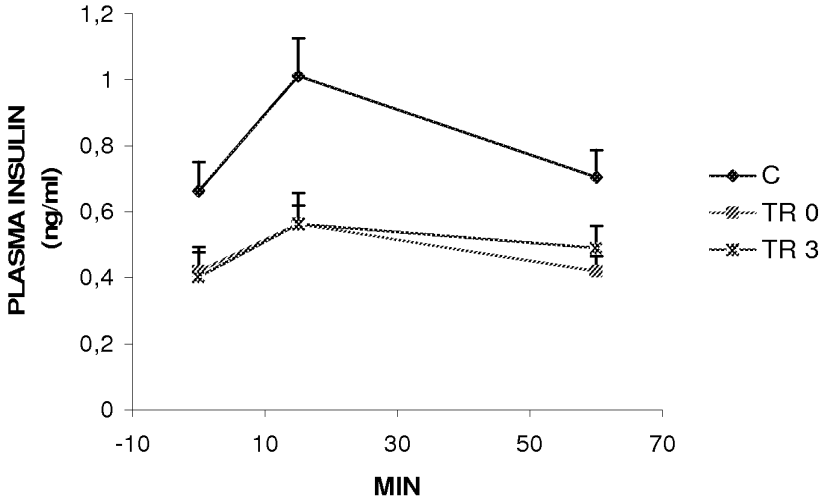
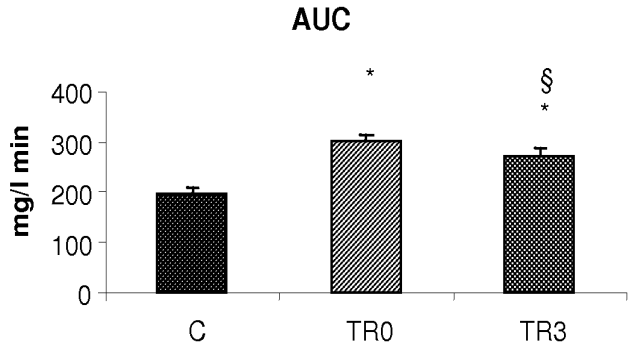
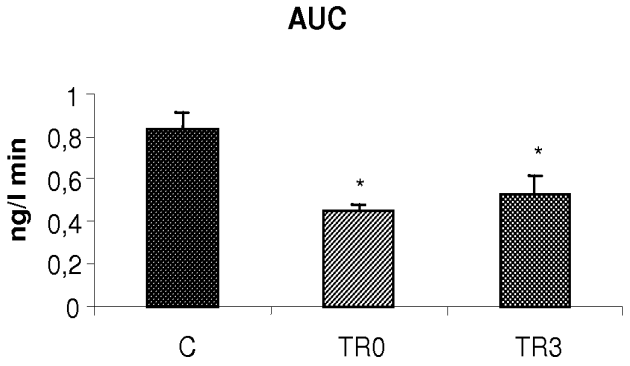
RESEARCH & PRODUCTION S.r.l.

PLASMA GLUCOSE and PLASMA INSULIN LEVELS after GTT









C = Control
TR0 = STZ-NA
TR3 = STZ-NA + IAC 30 mg/Kg

* p<0.05 vs. CONTR

§ p<0.05 vs. STZ-NA

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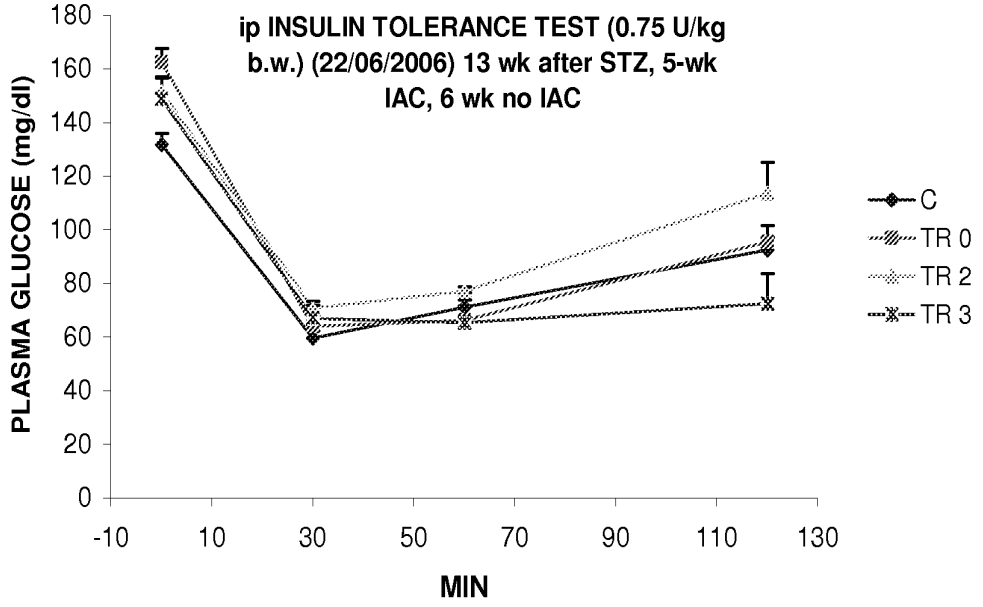
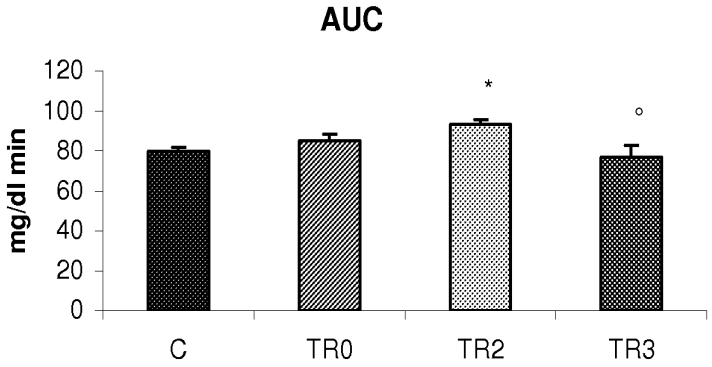
PLASMA GLUCOSE LEVELS
after Insulin Tolerance Test



C = Control
TR0 = STZ-NA
TR2 = STZ-NA + IAC 15 mg/kg
TR3 = STZ-NA + IAC 30 mg/Kg



* p<0.05 vs. CONTR

° p<0.05 vs. STZ-NA

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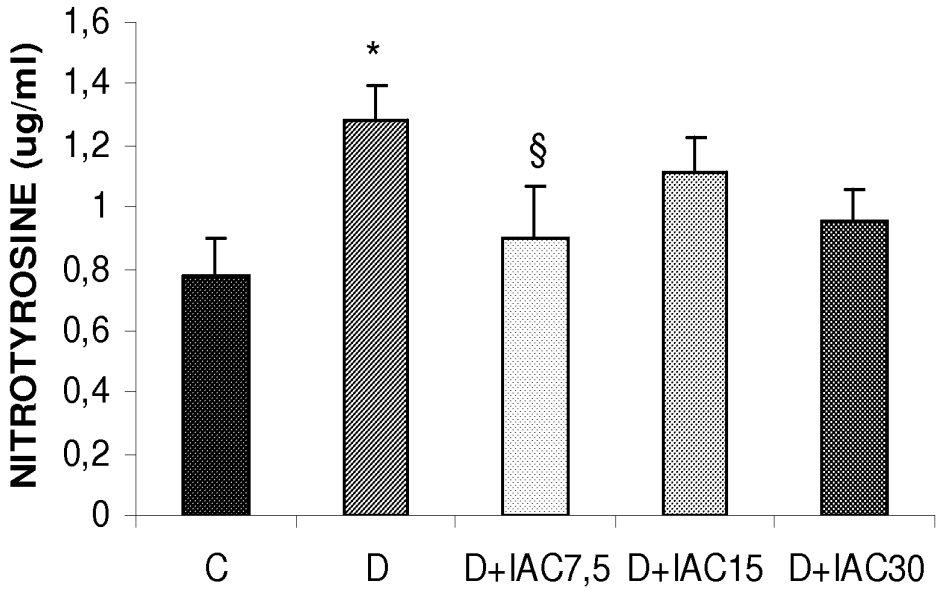
PLASMA NITROTYROSINE LEVEL

PLASMA NITROTYROSINE LEVELS AFTER SUSPENSION OF IAC



C = Control
D = STZ-NA
D+IAC7,5 = STZ-NA + IAC 7,5
D+IAC15 = STZ-NA + IAC 15
D+IAC30 = STZ-NA + IAC 30

* p<0.05 vs. CONTR

§ p<0.05 vs. STZ-NA

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CONCLUSIONS

In the STZ-NA diabetic mouse model IACVITA improves diabetic metabolic alterations, likely by counteracting beta-cell dysfunction and loss associated with oxidative stress.

In addition, IACVITA treatment (15 or 30 mg/kg per day IP) induced no apparent toxic effect.

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Reduction of Oxidative Stress by a New Low-Molecular-Weight Antioxidant Improves Metabolic Alterations in a Nonobese Mouse Diabetes Model

Michela Novelli, PhD, Valentina D'Aleo, PhD,* Roberto Lupi, PhD,† Moreno Paolini, PhD,‡ Antonio Sodi, PhD,‡ Piero Marchetti, MD, PhD,† and Pellegrino Masiello, MD**

Objectives: We have previously established a nonobese diabetic mouse model characterized by moderate hyperglycemic levels, like those usually occurring in human type 2 diabetes. As oxidative stress is considered a major mechanism of progressive β-cell damage in diabetes, we aimed in this model the protective effects of a new low-molecular-weight antioxidant, namely, *bis*(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decanedioic dihydrochloride (IAC).

Methods: Diabetes was induced in CS7BL6J mice by streptozotocin (STZ) and nicotinamide (NA) administration. Two weeks later, STZ-NA mice were treated for 5 weeks with different doses of IAC (15 or 30 mg/kg per day intraperitoneally) and monitored for glycemia, insulinemia, glucose tolerance, and pancreatic insulin content.

Results: Streptozotocin-NA mice showed moderate hyperglycemia, hypoinsulinemia, glucose intolerance, growth impairment, and markedly reduced pancreatic insulin content (22% of controls). IAC-treated STZ-NA mice showed dose-related reduction of hyperglycemia and attenuation of glucose intolerance, associated to higher residual pancreatic insulin content with respect to untreated diabetic animals. Plasma nitrotyrosine levels (an index of oxidative stress), enhanced 3-fold in diabetic mice, were significantly reduced by IAC treatment. Significant correlations were found between plasma nitrotyrosine values and either blood glucose levels or pancreatic insulin content.

Conclusions: In the STZ-NA diabetic mouse model, the new antioxidant, IAC, improves diabetic metabolic alterations, likely by counteracting β-cell dysfunction and loss associated with oxidative stress.

Key Words: type 2 diabetes, oxidative stress, antioxidant treatment, nitrotyrosine, pancreatic insulin content, mouse

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From the *Dipartimento di Patologia Sperimentale and †Dipartimento di Endocrinologia e Metabolismo, University of Pisa, Pisa; ‡Dipartimento di Farmacologia, University of Bologna, Bologna; and §Medestea Research, Turin, Italy.

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Reprints: Pellegrino Masiello, MD, Dipartimento di Patologia Sperimentale BMIE, University of Pisa, Via Roma, 55 Scuola Medica, 56126 Pisa, Italy (e-mail: p.masiello@med.unipi.it).

Type 2 diabetes (T2D) is characterized by insulin secretory dysfunction associated with variable degrees of peripheral insulin resistance.[1] Usually, the disease arises because of the progressive failure of β cells to adequately match insulin secretion to the increased insulin demand in insulin-resistant states. Indeed, prospective studies have clearly demonstrated that the progressive nature of diabetes is an ongoing decline in β-cell function without a change in insulin sensitivity.[2,3] This relative functional impairment could be due to an intrinsic secretory defect of the β cells or to reduced β-cell mass, or both. Among the various mechanisms that have been proposed to be responsible for the β-cell decompensation, oxidative stress is considered to play a major role not only in the accelerated functional demise but also in the progressive loss of β cells.[4,5] Oxidative stress is also known to be involved in the pathogenesis of diabetic vascular complications, both microvascular and macrovascular.[6]

Actually, in T2D, increased plasma glucose levels, often associated with hypertriglyceridemia, result in enhanced mitochondrial superoxide production, with consequent increased exposure of cells to reactive oxygen species (ROS),[7–9] which could also be generated by the nonenzymatic glycosylation reaction[10] and the hexosamine pathway.[11]

Reactive oxygen species are generally considered to be strongly implicated in damage of various cell types, including endothelial and pancreatic β cells, acting by various mechanisms such as activation of poly(ADP-ribose) synthetase[12] and lipid peroxidation.[13] Moreover, the enhanced ROS production can induce inducible nitric oxide (NO) synthase expression and thus NO synthesis.[14] The simultaneous overgeneration of NO and superoxide favors the production of the cytotoxic peroxynitrite anion, which oxidizes sulfhydryl groups in proteins, initiates or aggravates lipid peroxidation, and nitrates amino acids such as tyrosine, possibly leading to major pathological consequences.[15] These ROS-induced cytotoxic effects might underlie the ongoing reduction in β-cell number associated with the progression of T2D[16,17] and the consequent failure of functional compensatory mechanisms able to counteract insulin resistance in both early and late phases of the disease.[18] It should also be considered that β cells are particularly sensitive to ROS excess because the expression of ROS-detoxifying enzymes in these cells is particularly low in comparison with other cell types.[19]

Therefore, to prevent or at least reduce β-cell functional impairment and death in T2D, it appears worthwhile to search for antioxidant agents capable of effectively inhibiting ROS action and limiting the cytotoxic chronic oxidative stress

IN VIVO IAC STUDY

- Niddm mice model
- Diabetes inducted with STZ + NA
- 5 groups (8 mice/group)
  - Control
  - STZ-NA
  - STZ-NA + IAC 7.5 mg/Kg
  - STZ-NA + IAC 15 mg/Kg
  - STZ-NA + IAC 30 mg/Kg

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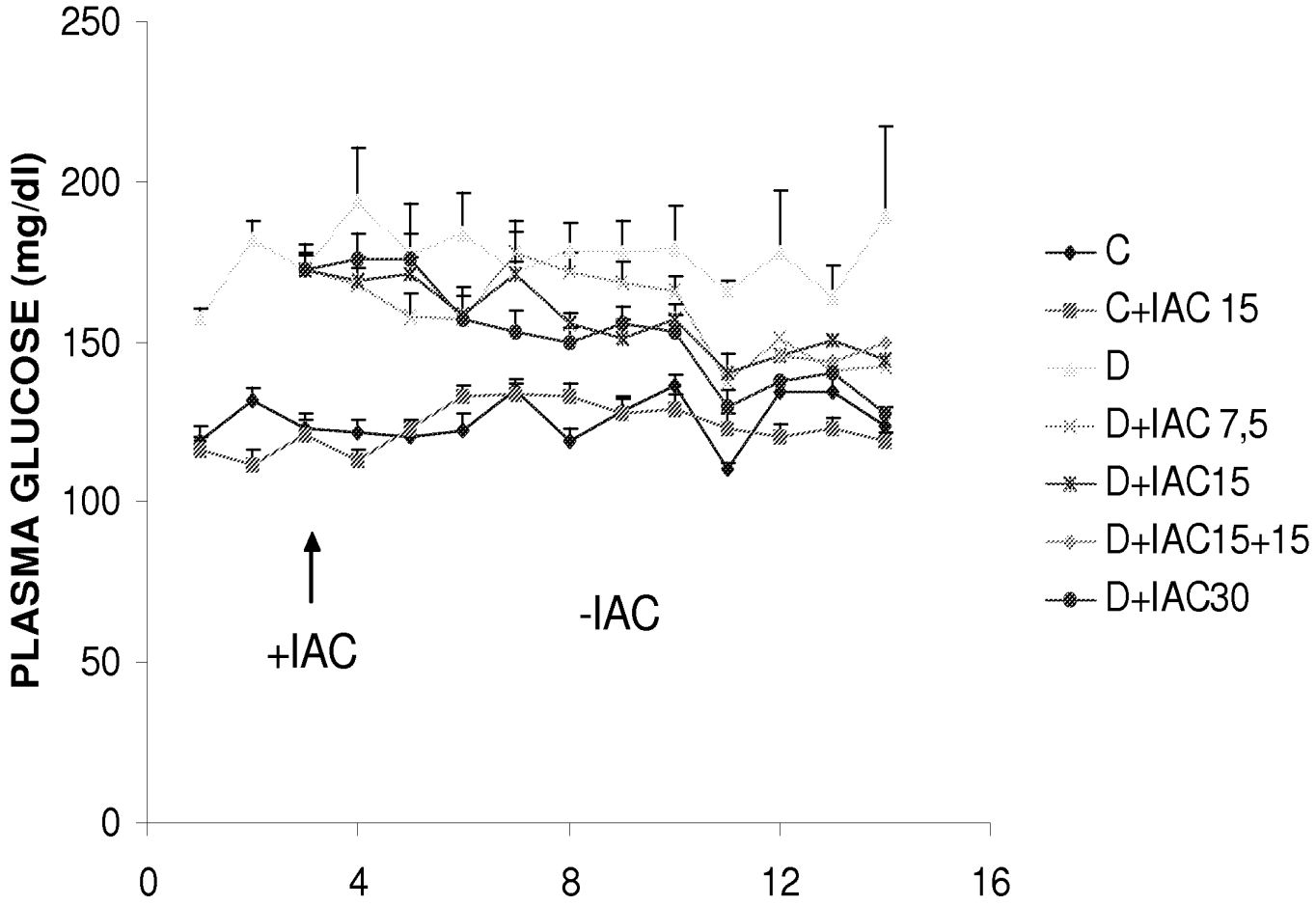
PLASMA GLUCOSE LEVELS



C =
C+IAC15 = Control + Iac 15 mg/kg
D = STZ-NA
D+IAC7,5 = STZ-NA + IAC 7,5 mg/Kg
D+IAC15 = STZ-NA + IAC 15 mg/Kg
D+IAC30 = STZ-NA + IAC 30 mg/Kg

MEDESTEA
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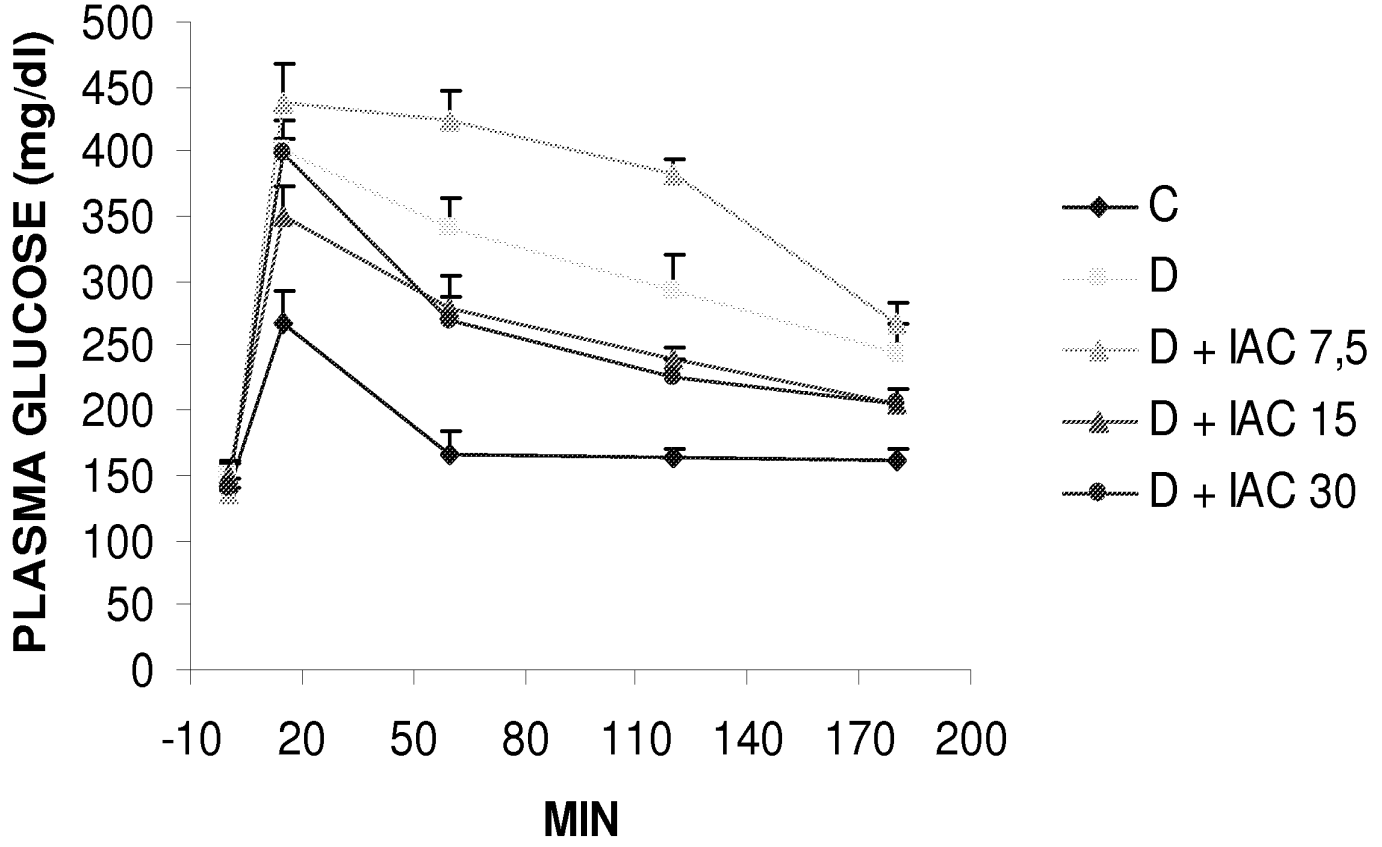
PLASMA GLUCOSE LEVELS



C = Control
D = STZ-NA
D+IAC7,5 = STZ-NA + IAC 7,5 mg/Kg
D+IAC15 = STZ-NA + IAC 15 mg/Kg
D+IAC30 = STZ-NA + IAC 30 mg/Kg

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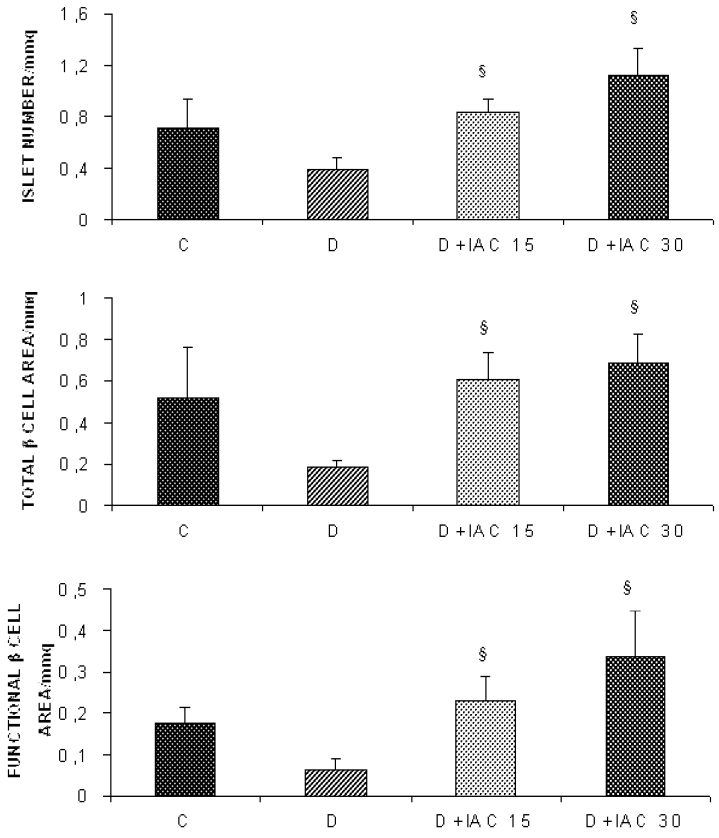
Evaluation of Histological and immunohistochemical features of Pancreatic islets:

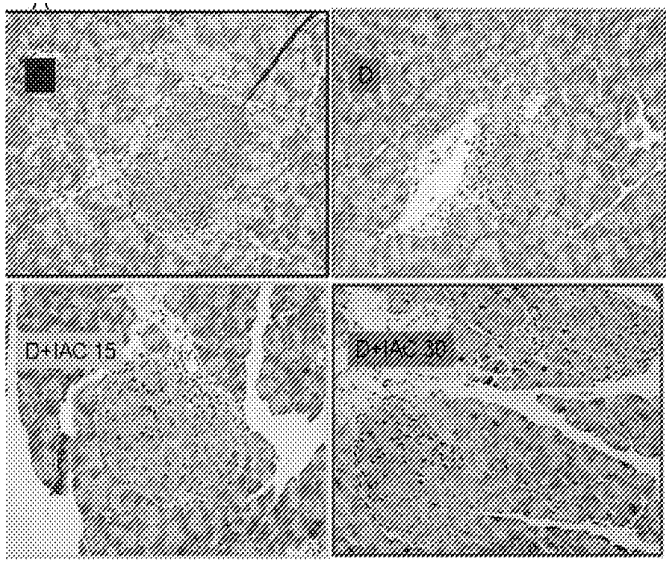
Morphometric analysis of islet number, total ß-cells area and functional ß-cells area

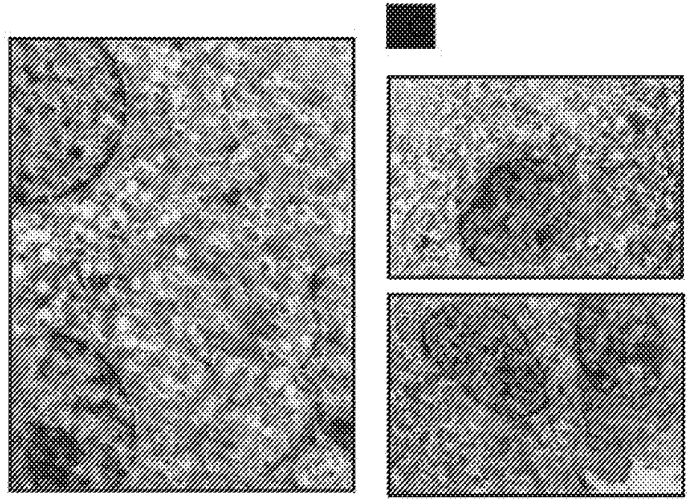


C: Healthy controls

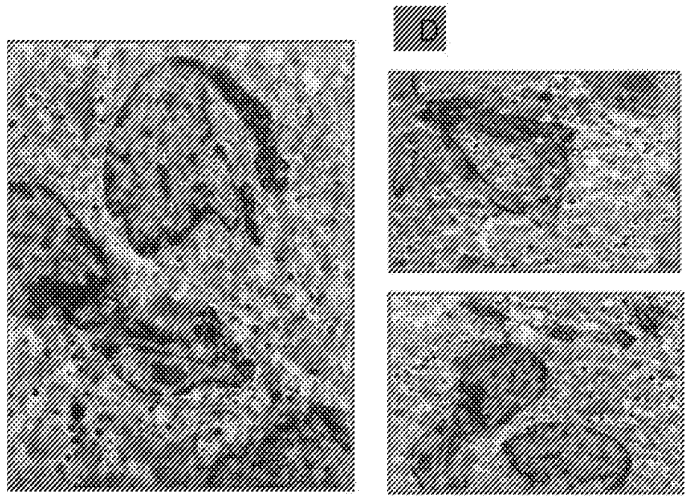
D: Untreated STZ-NA diabetic mice

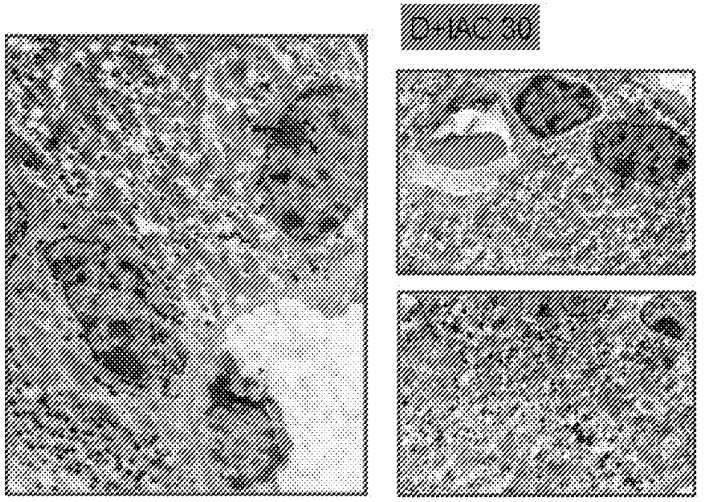
Anti-insulin staining of pancreas from control and diabetic mice





Representative electron microscope images of controls (C), untreated diabetic mice (D) and diabetic mice treated with Iac 30mg/Kg/day (D+ IAC30)





CONCLUSIONS

In the STZ-NA murine model of diabetes, the new antioxidan compaund lacvita was able not only to counteract B-cells dysfunction and loss associated with oxidative stress but also to reconstitute B-cells population. Studies are in progress to clarify the underlying mechanisms of such findings.

CONCLUSIONS

IAC showed a strong level of activity on Langherans islets from either animal and human

1) In human Langherans islets, “poisoned” with concentrated glucose and FFA, IAC could restore in vitro their correct sensitivity to glucose and their ability to produce a rate of insulin close to normality;

2) In the case of human biopsies of Langherans islets from dead type II diabetes patients, IAC was able to “reset” their activity from the actual 30% up to 70% of the normal rate;

3) In animal type II Diabetes model (treatment with STZ-NA), IAC was able to reduce the glicemia close to the normality in 5 weeks of treatment. The effect lasted for the 5 following weeks of follow up without treatment.

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OVERALL CONCLUSIONS

1) In human Langherans islets, "poisoned" with concentrated glucose and FFA, IACVITA could restore in vitro their correct sensitivity to glucose and their ability to produce a rate of insulin close to normality;

2) In the case of human biopsies of Langherans islets from dead type II diabetes patients, IACVITA was able to "reset" their activity from the actual 30% up to 70% of the normal rate;

3) In animal type II Diabetes model (treatment with STZ-NA), IACVITA was able to reduce the glicemia close to the normality in 5 weeks of treatment. The effect lasted for the 5 following weeks of follow up without treatment.

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Abstract published on IACVITA in the treatment of diabetes

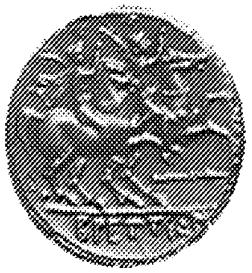
- R. Mancarella, R. Lupi, S. Del Guerra, M. Novelli, M. Bugliani, S. Torri, L. Valgimigli, GF Pedulli, M. Paolini, A. Soleti, M. Galli, V. D'Aleo, F. Filipponi, F. Mosca, U. Boggi, S. Del Prato, P. Masiello, P. Marchetti ***"THE EFFECT OF THE ANTIOXIDANT MOLECULE IACVITA ON THE OXIDATIVE STRESS OF CULTURED HUMAN ISLETS"***

- R. Lupi, R. Mancarella, S. Del Guerra, M. Masini, A. Soleti, M. Paolini, M. Martano, M. Bugliani, S. Torri, M. Galli, V. D'Aleo, M. Del Chiaro, S. Del Prato, U. Boggi, F. Filipponi, Piero Marchetti ***"BENEFICIAL EFFECT OF THE NON-PEPTIDYL LOW MOLECULAR WEIGHT RADICAL SCAVENGER IACVITA ON CULTURED HUMAN ISLET FUNCTION"***

- S. Del Guerra, R. Lupi, A. Soleti, F. Riccardino, M. Paolini, R. Mancarella, M. Bugliani, S. Torri, M. Galli, V. D'Aleo, U. Boggi, F. Filipponi, S. Del Prato, F. Mosca, P. Marchetti ***"LIPOTOXICITY IN HUMAN PANCREATIC ISLETS IS MEDIATED BY OXIDATIVE STRESS: EVIDENCE OF THE PROTECTIVE ROLE OF A NON-PEPTIDYL LOW MOLECULAR WEIGHT RADICAL SCAVENGER (IACVITA)"***

IAC cardio

EXHIBIT 4



University of Catanzaro "Magna Graecia"

Faculty of Pharmacy

*The protective effect of **IAC** on balloon injury-related neointima formation.*

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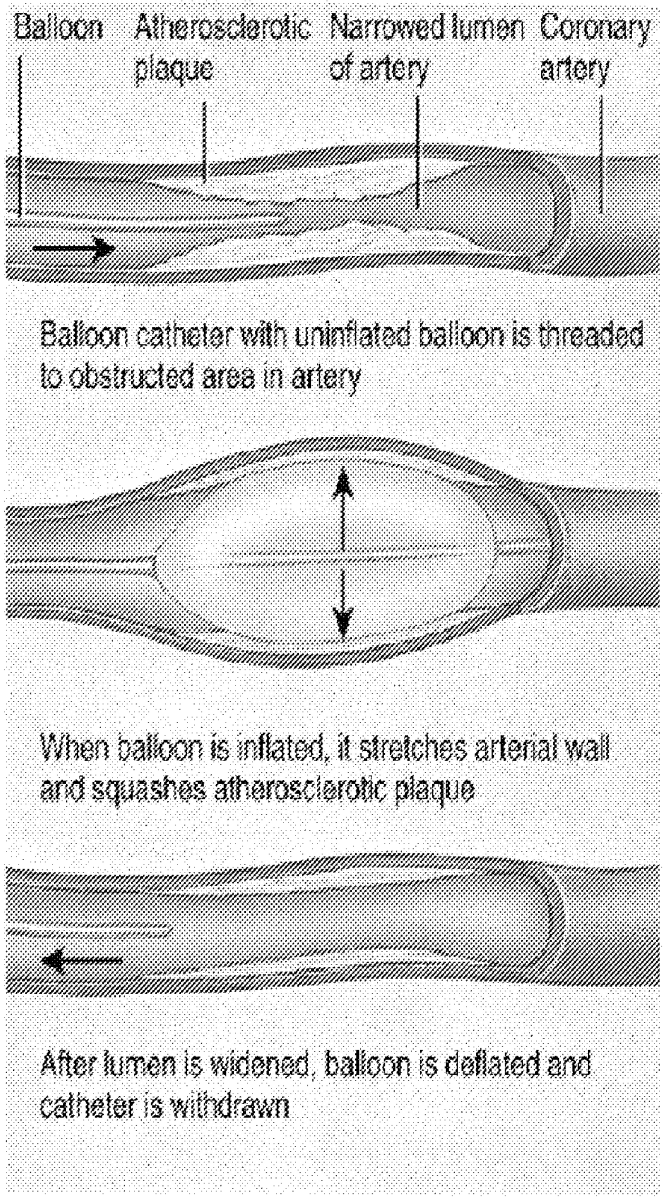
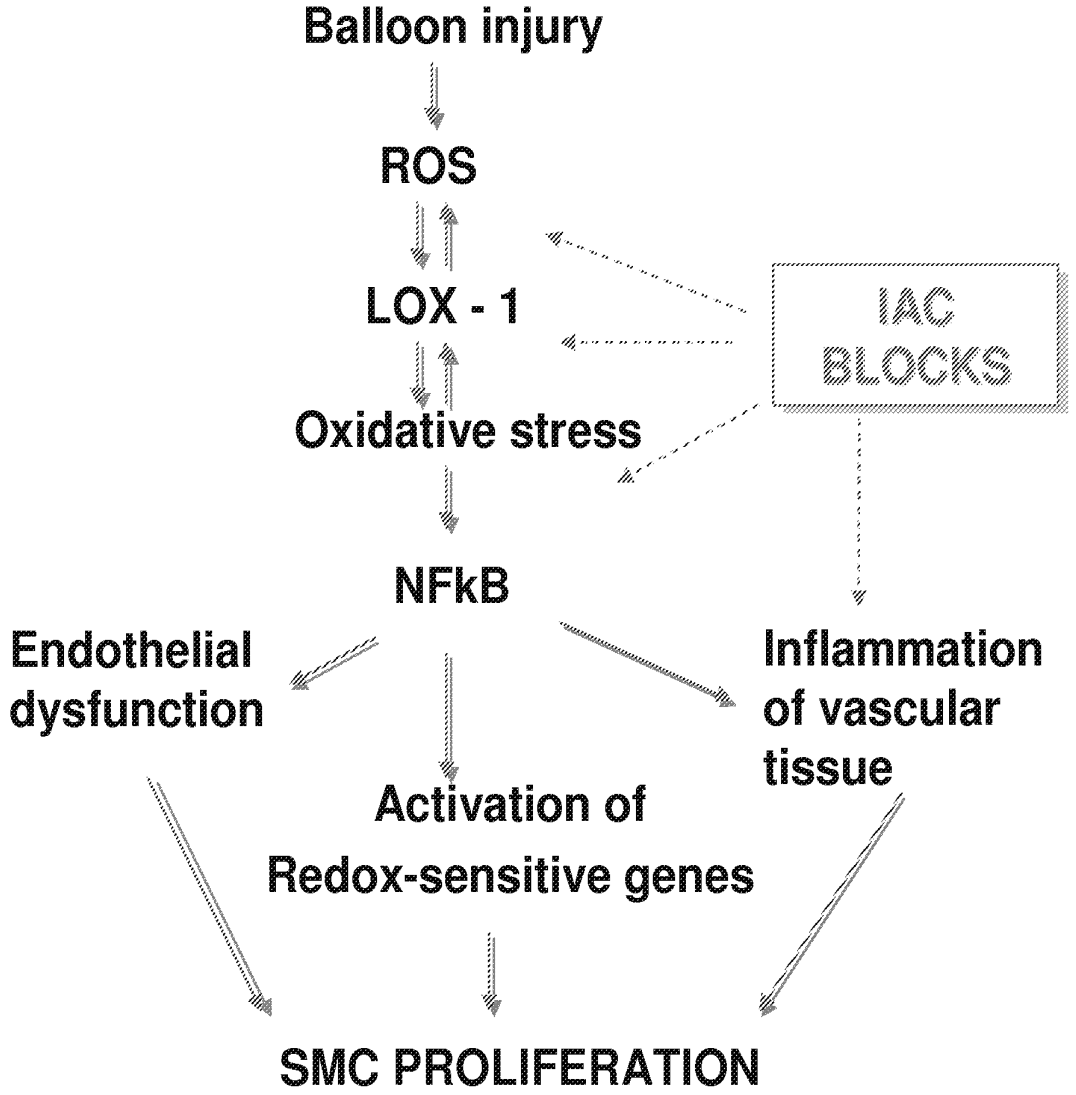
Carotid artery was injured using a balloon embolectomy catheter.





MEDESTEA
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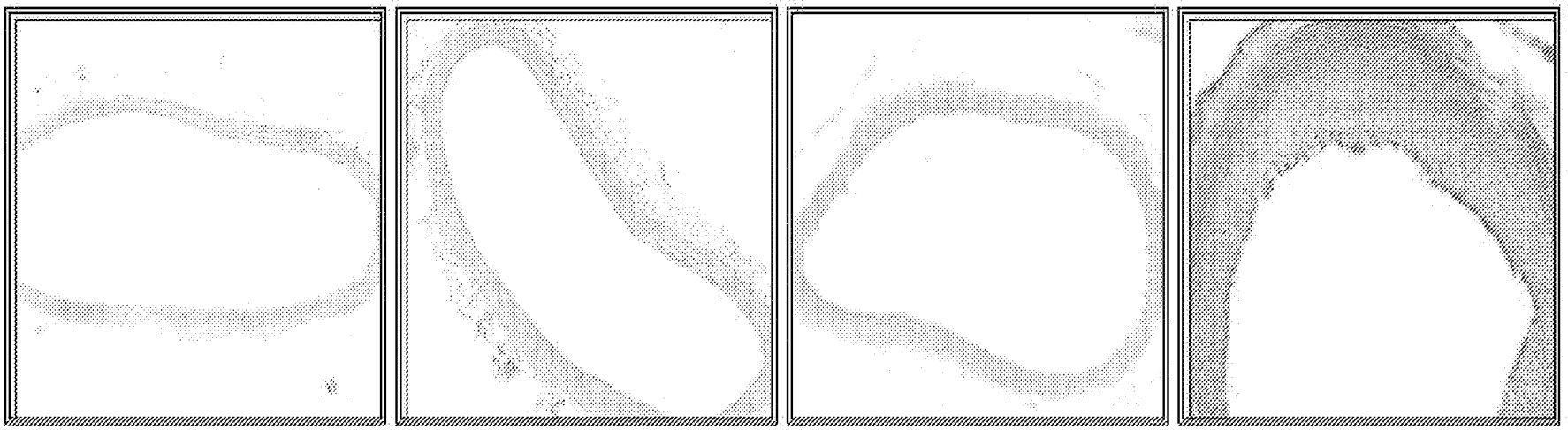
Proliferation of sub-endothelial vascular smooth muscle cells (SMCs) after *balloon injury*.



CTRL | 4 days post injury | 7 days post injury | 14 days post injury

MEDESTEA
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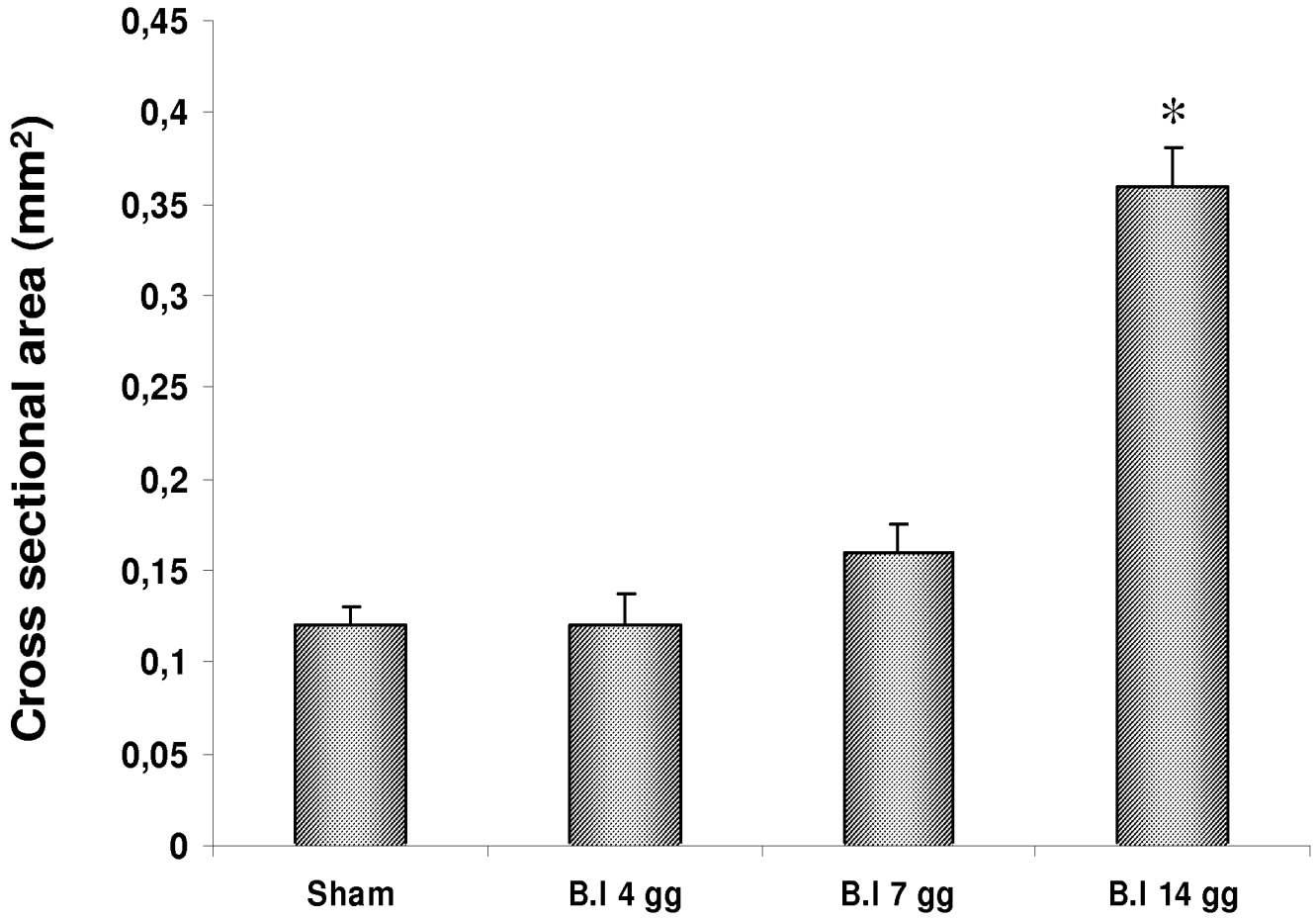
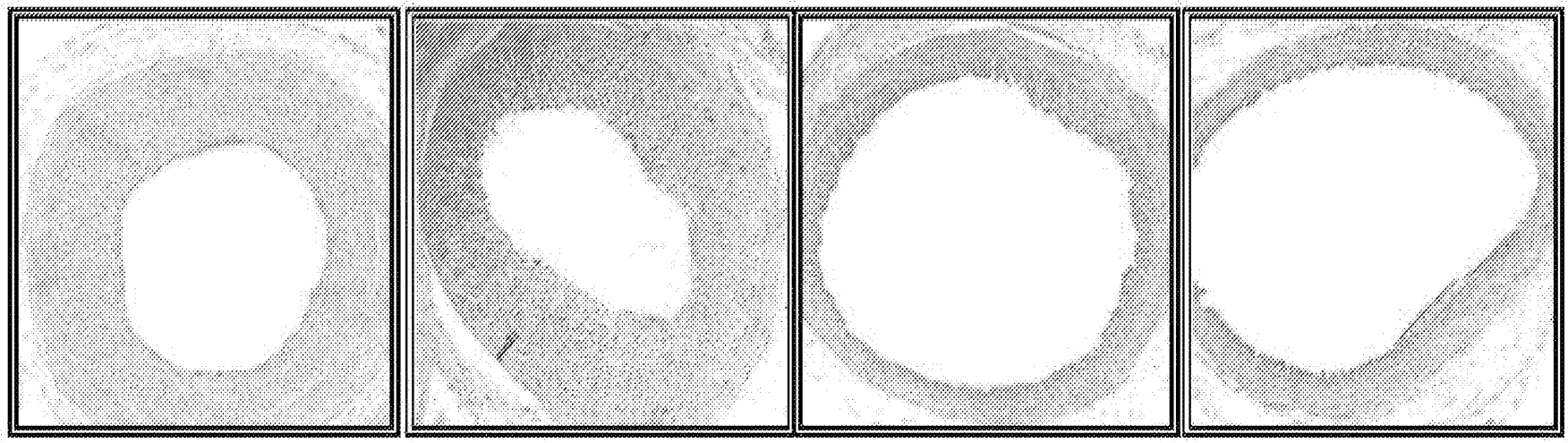
Proliferation of sub-endothelial vascular smooth muscle cells (SMCs) after *balloon injury*.



MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Daily i.p. treatment of rats with IAC significantly antagonizes dose dependently ballon-induced neointima formation.



Injury 14 gg

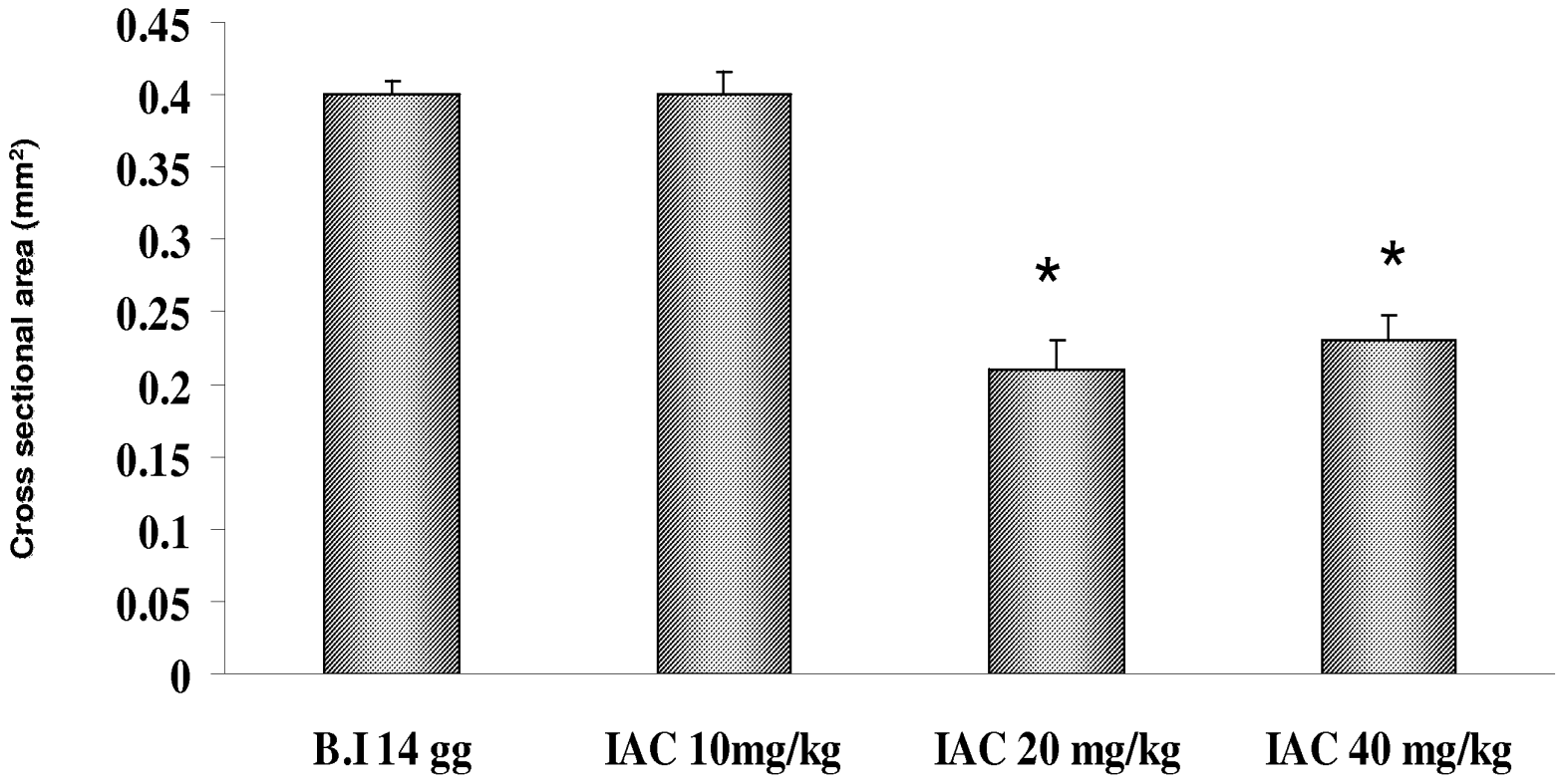
Injury 14 gg + IAC 10 mg/kg

Injury 14 gg + IAC 20 mg/kg

Injury 14 gg + IAC 40mg/Kg

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

Daily i.p. treatment of rats with IAC significantly antagonizes dose dependently ballon-induced neointima formation.



* $P < 0.001$ when compared *vs* injury

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CONCLUSIONS

1) In rats undergoing balloon injury of left carotid artery, a significant vascular SMCs proliferation occurred when compared to sham operated animals.

2) Early phases of neointima formation were characterized by an intense production of reactive oxygen species.

3) Treatment of rats with IAC i.p., the most powerful safe free radical scavanger known today (500 times stronger than DMPO), significantly antagonizes balloon-injury neointima formation. Indeed, both cross sectional area of injured carotid artery and intima/media ratio were reduced dose-dependently by daily administration of IAC.

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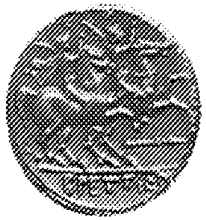

University of Catanzaro "Magna Graecia"

Faculty of Pharmacy

The protective effect of IAC on cardiac ischemia: ischemia-reperfusion in the isolated perfused Langerdorff heart.

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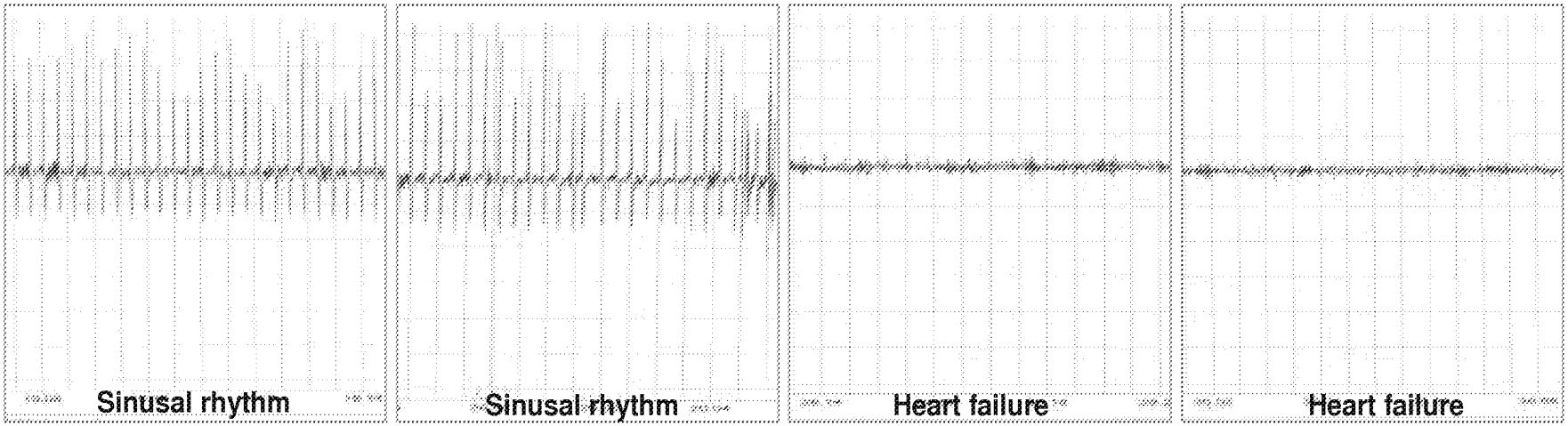
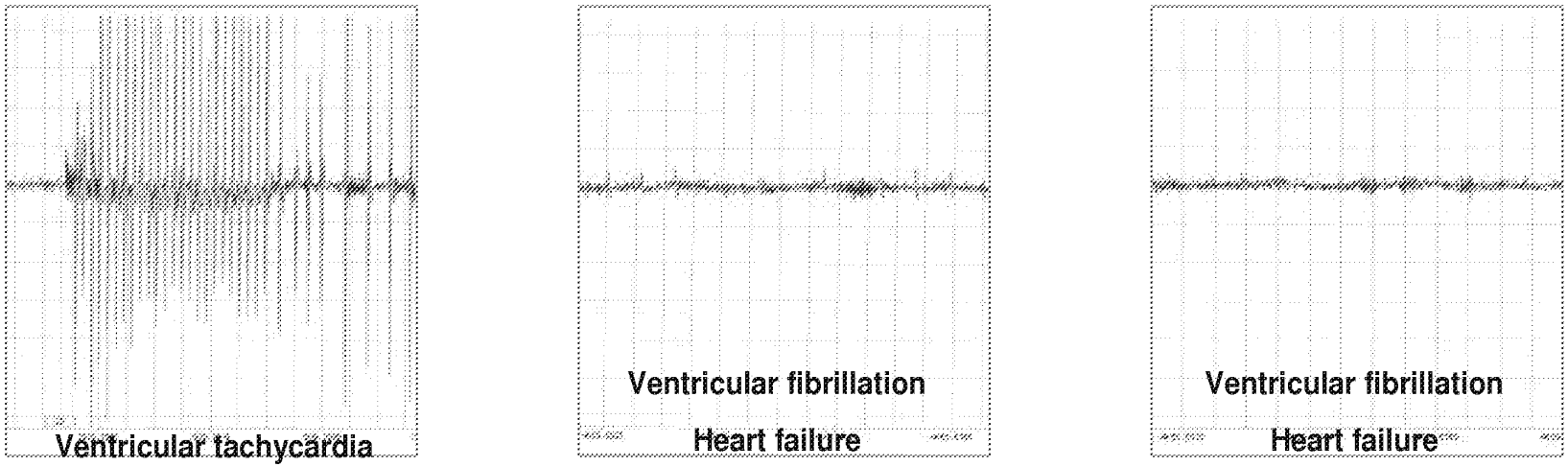
EXHIBIT 5

ISCHEMIA 15 MINUTES



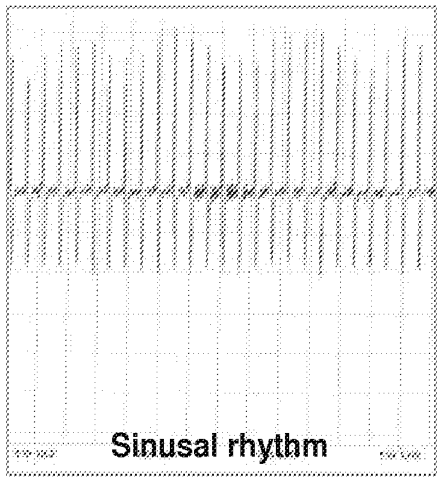
Basal | Ischemia 0' | Ischemia 5' | Ischemia 10'



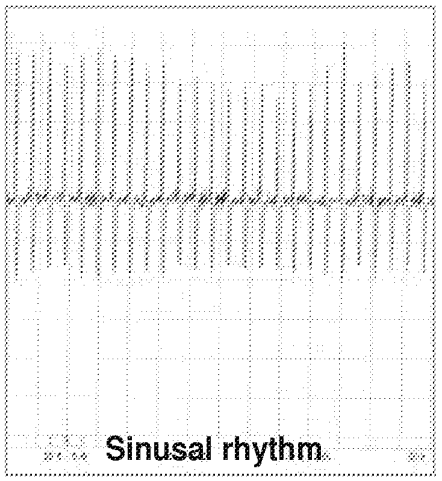
Riperfusion 0' | Riperfusion 5' | Riperfusion 10'

MEDESTEA
RESEARCH & PRODUCTION S.r.l.

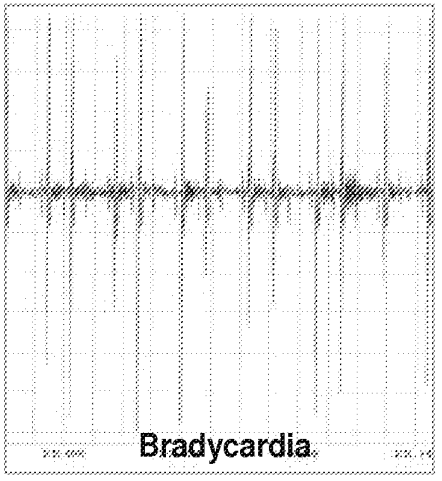
ISCHEMIA 15 MINUTES + IAC 10 MicrMol



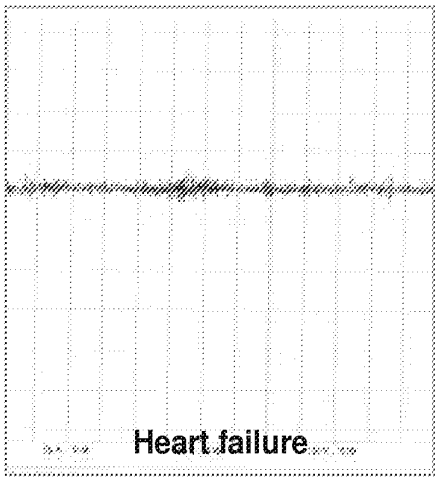
Basal



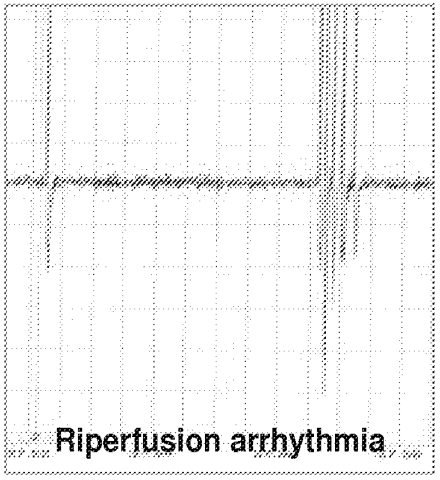
Ischemia 0’



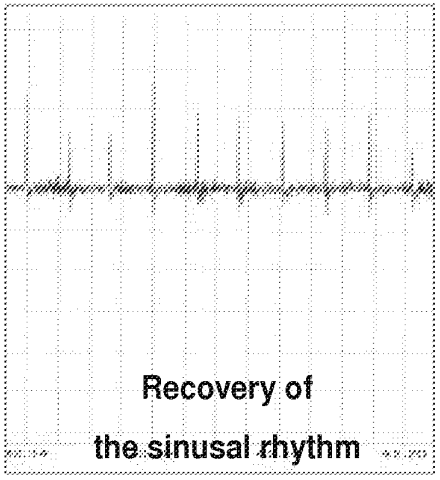
Ischemia 5’



Ischemia 10’



Riperfusion 0’



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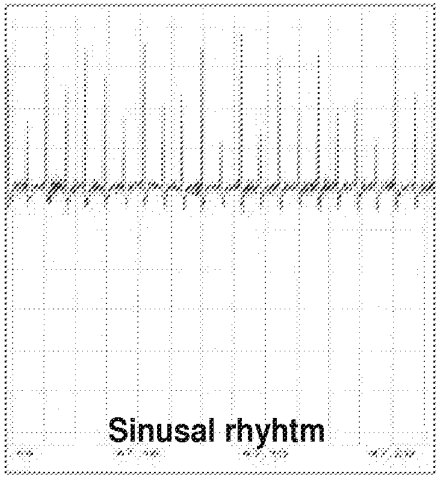

Riperfusion

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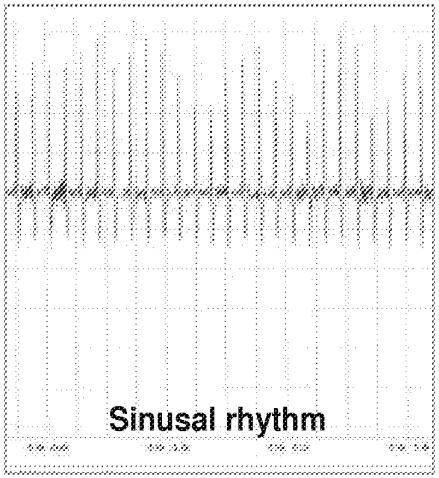
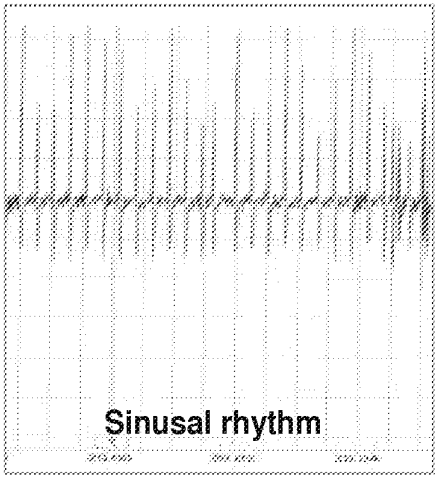
ISCHEMIA 15 MINUTES + IAC 50 MicrMol



Basal



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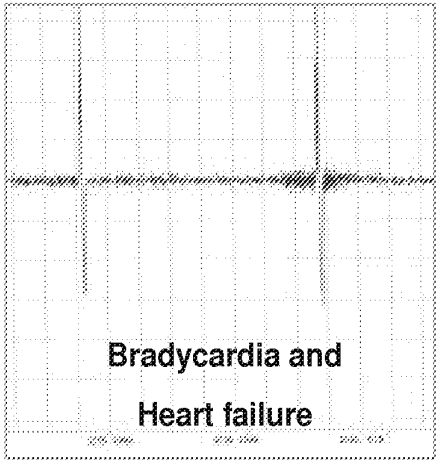

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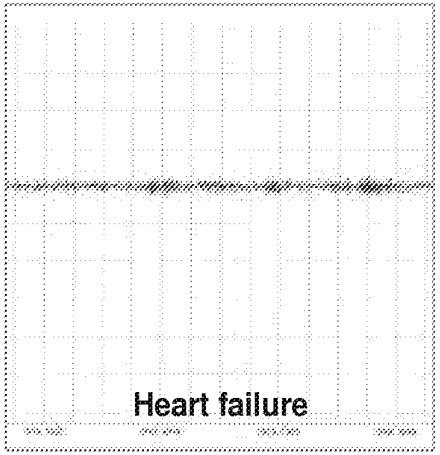
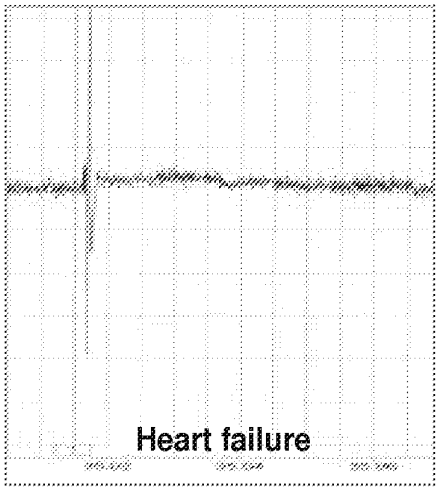
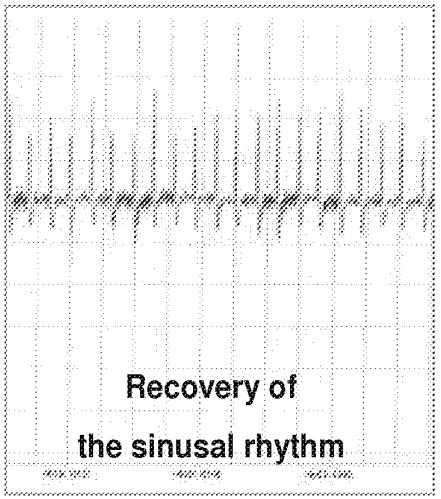


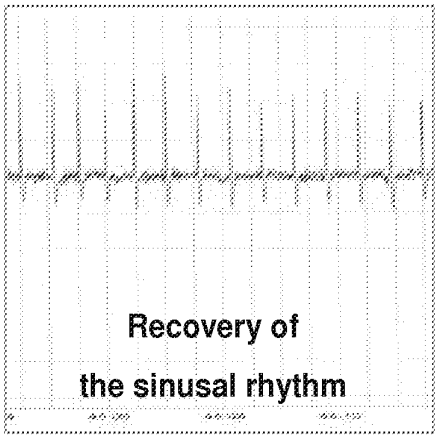
Ischemia 10'



Riperfusion 0'



Riperfusion 5'



Riperfusion 10'

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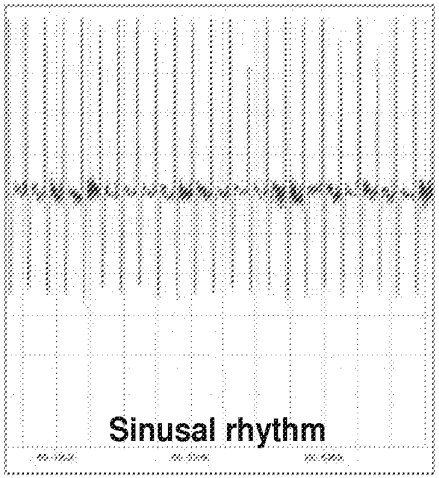
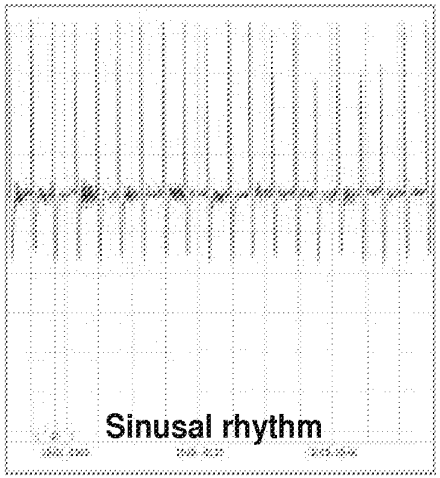
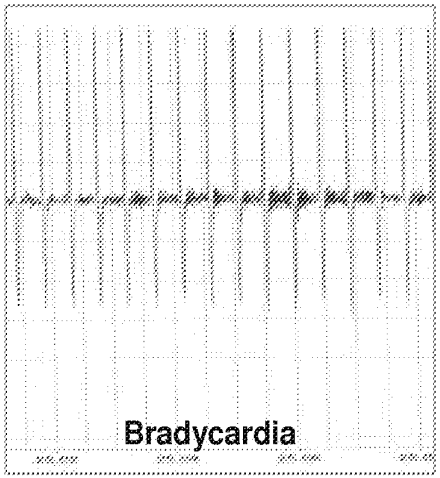
ISCHEMIA 15 MINUTES + IAC 100 MicrMol



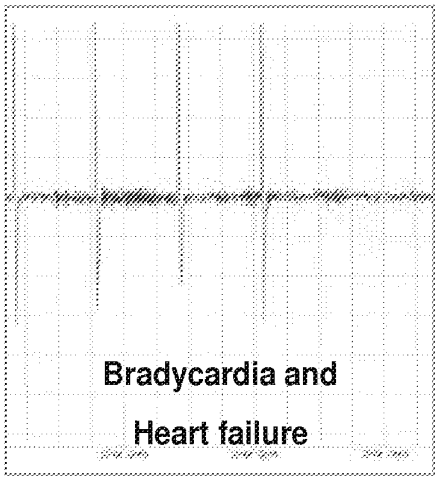
Basal



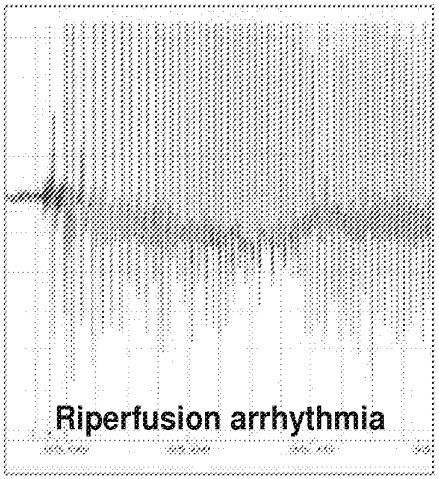
Ischemia 0’



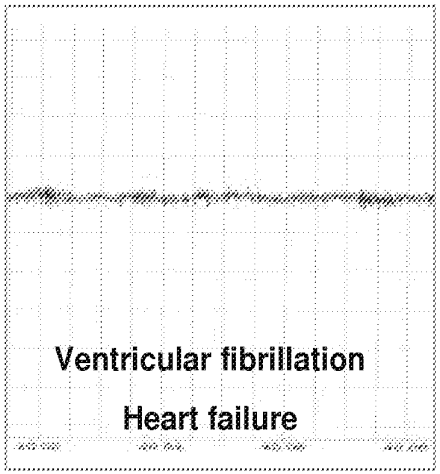
Ischemia 5’



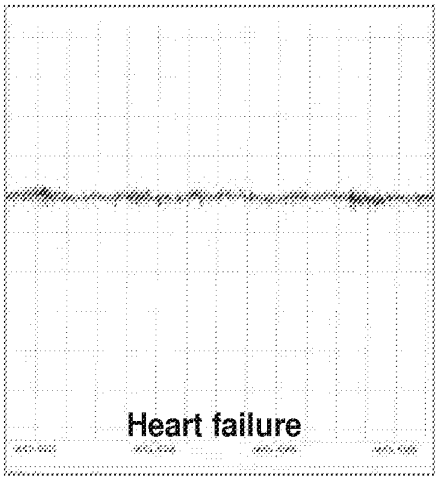
Ischemia 10’



Riperfusion 0’



Riperfusion 5’



Riperfusion 10’

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ISCHEMIA 15 MINUTES

Ischemia 15 min	Duration of post ischemic heart failure(min)	BEV first 3 min	Ventricular fibrillation	Duration of heart failure
CTRL	4,41 + 0,3	78 + 6	100%	2
IAC 10	3,88 + 0,6	62 + 5	0	-
IAC 50	4,98 + 0,5	32 + 6	0	-
IAC 100	8,17 + 0,3	55 + 3	25%	8

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PharmaHungary.com

PHARMAHUNGARY™

A leader in the non-clinical screening of drug candidates focusing on cardiovascular diseases.

Effects of **IAC** on Myocardial Ischemia and reperfusion on rat.

EXHIBIT 6

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Study outline

- Male Wistar rats (total N=60)
- 30 min regional ischemia (occlusion of the coronary artery)
- 120 min reperfusion
- 5 groups i.v. immediatly before and 60 min after reperfusion:
  - Veichle
  - IAC: 0.1, 0.4, 2 and 8 mg/kg
- Evaluation of:
  - Infarct size (TTC/Evans blue method)
  - CK, LDH, SOD and MDA
  - ECG, blood pressure and temperature
  - Incidence of ventricular fibrillation during reperfusion
  - Mortality

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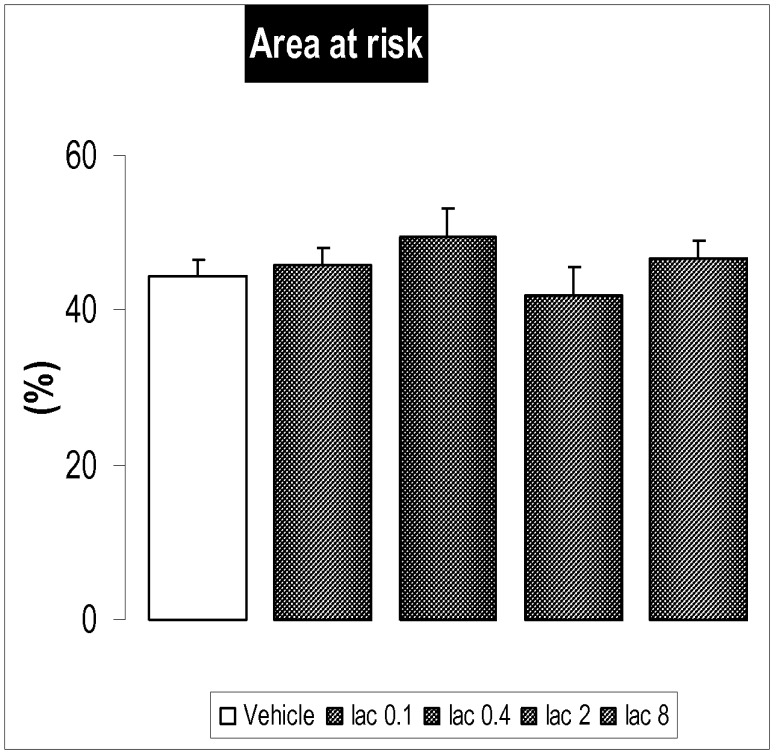
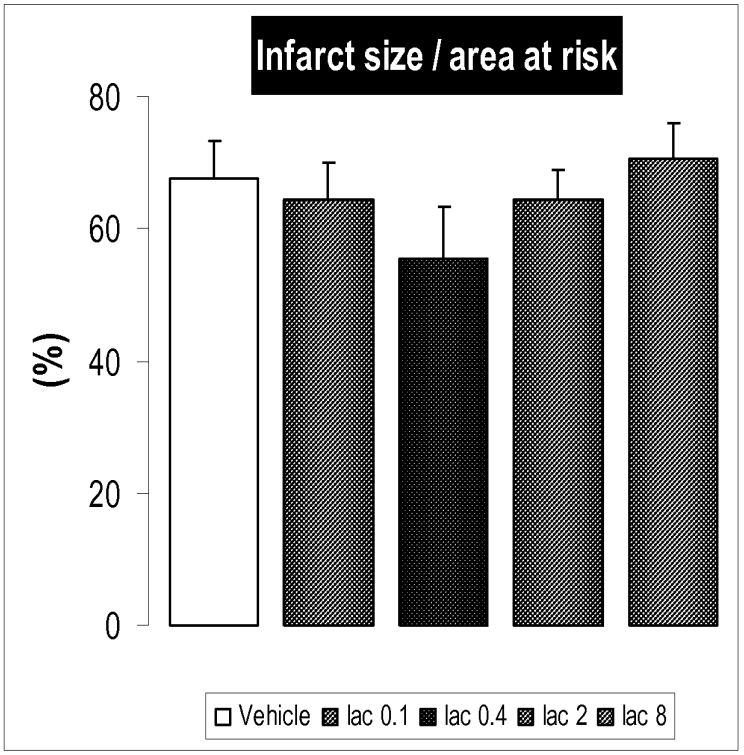
Results





	Vehicle	lac 0.1	lac 0.4	lac 2	lac 8
Incidence of VF	**2/15**	**1/13**	**3/12**	**2/12**	**2/13**
Incidence of VF (%)	13,3	7,7	25,0	16,6	15,4
mortality#	**0/15**	**1/13**	**1/12**	**0/12**	**1/13**
mortality (%)	0	7,7	8,3	0	7,7

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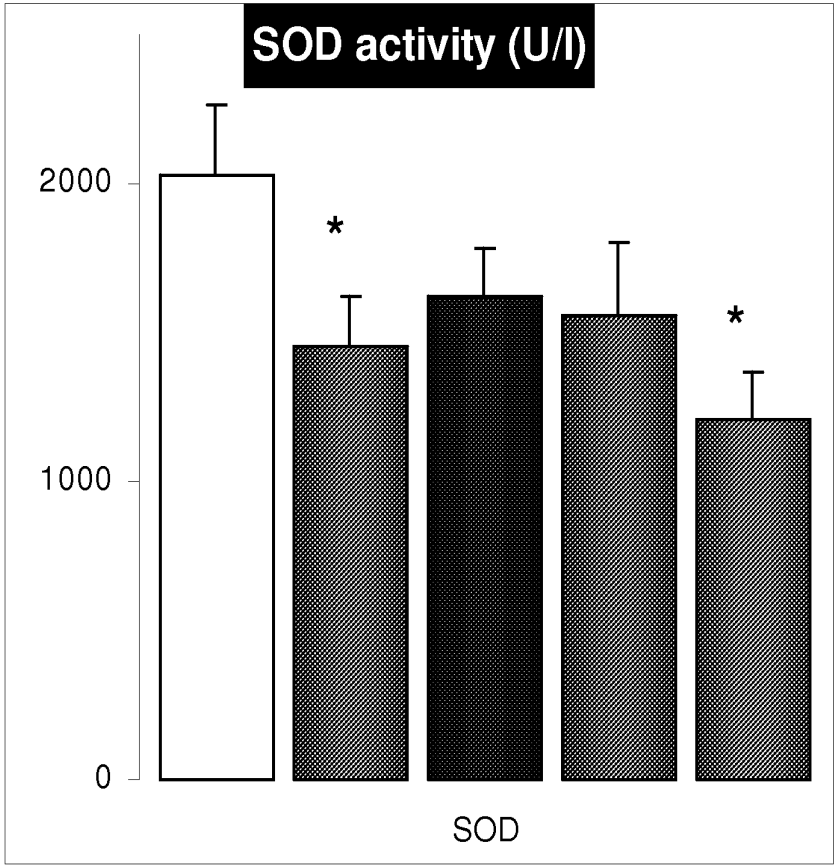
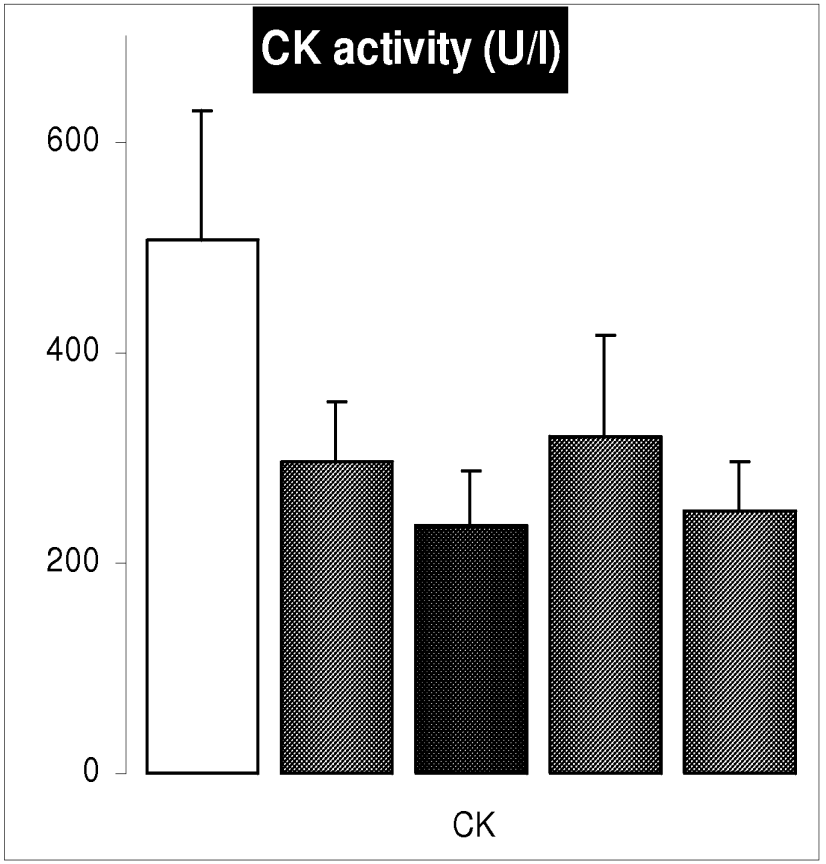
Results





* p<0.05

Vehicle
IAC 0.1
IAC 2
IAC 0.4
IAC 8

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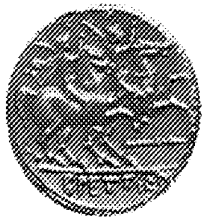

University of Catanzaro "Magna Graecia"

Faculty of Pharmacy

The anti-hypertensive activity of IAC.

EXHIBIT 7

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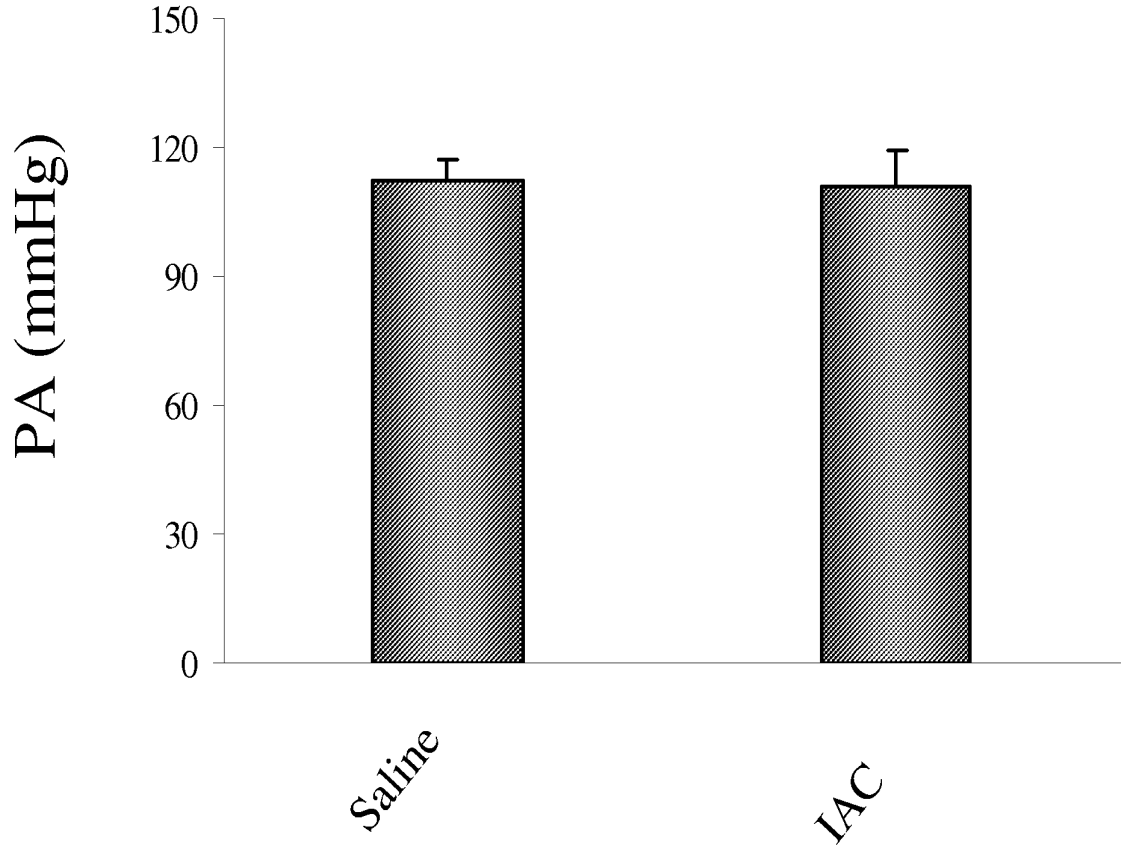
The ip administration of IAC (12mg/kg/die for 21days) doesn't change the PA value in Wistar normotensive rats.



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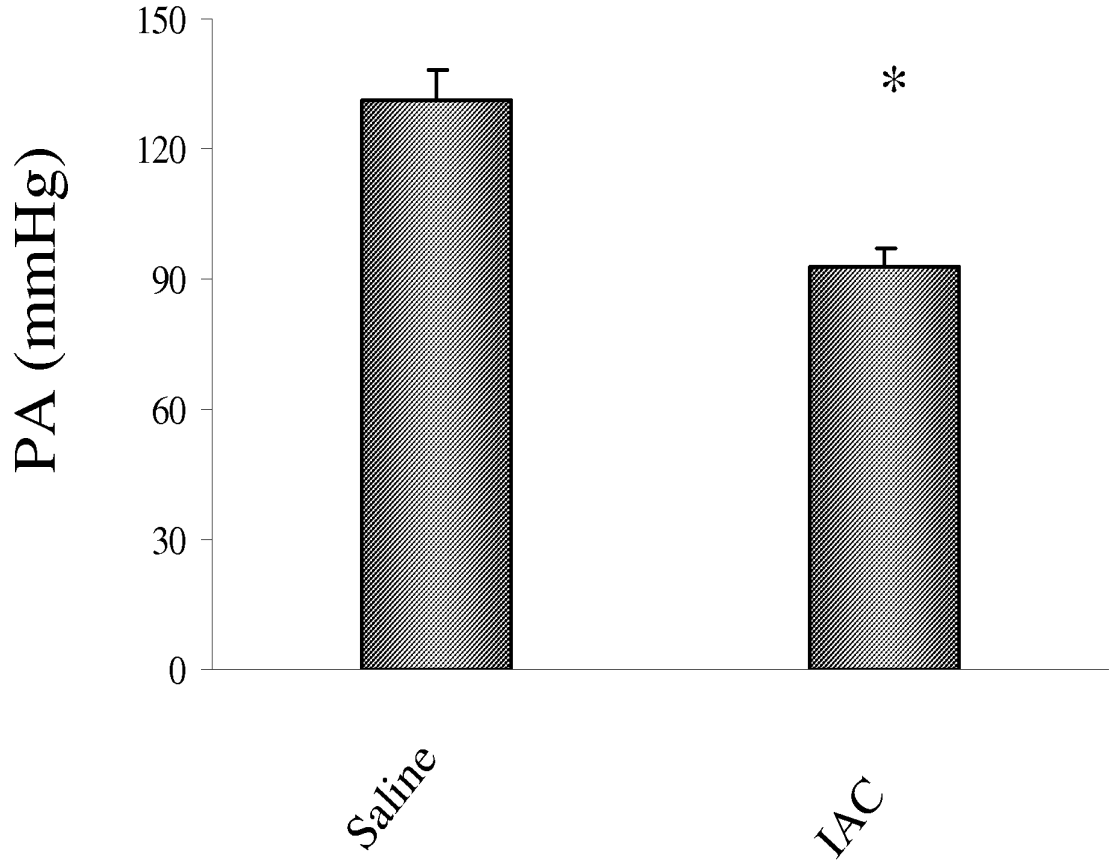
IAC ip treatment (12mg/kg/die for 21days) reduces basal PA in genetically hypertensive rats (SHR).



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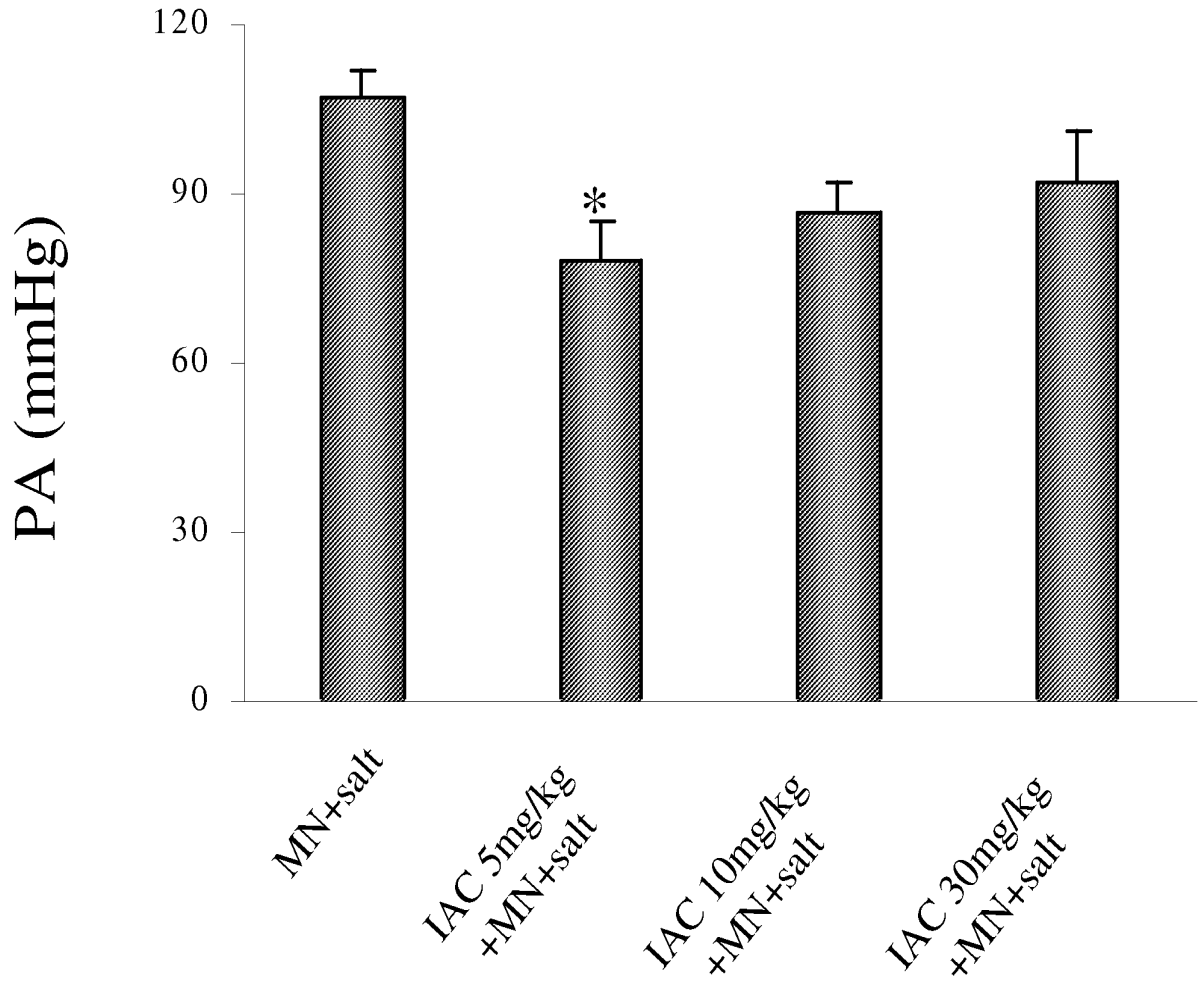
IAC activity on the variation of PA in mononefrectomized rats with a diet rich in salt.



* P < 0.05 when compared *vs* operated animals

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CONCLUSIONS

- An exaggerated production of superoxide by the vascular wall has been observed in different animal models of hypertension including spontaneously hypertensive rats.

- Between the VII and the XII weeks of life Spontaneously Hypertensive Rats move from a condition of borderline hypertension to stable hypertension.

- IAC treatment restores SHR blood pressure to normal value while it doesn't exert any hypotensive effect in Whistar normal rats indicating that IAC acts as an anti-hypertensive and not as a hypotensive drug.

- IAC treatment of mononefrectomized animals with a salt rich diet restores the blood pressure to normal level indicating that it is active also in the situation of hydrosaline retention.

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IAC mucositis

EXHIBIT 8

Biomodels
AND AFFILIATES

Biomodels, LLC and affiliates

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Study outline

In vivo Oral Mucositis Induced by Acute Radiation (40 Gy) in Hamsters

- 64 male Syrian Golden Hamster/LVG
- i.p. daily administration: from day -1 up to day 15 (see treatment scheme)
- Iacvita doses: 3 mg/kg and 30 mg/kg
- Mucositis scoring: validated photographic scale, ranging from 0 for normal, to 5 for severe ulceration (see score table)
- Mucositis evaluation: starting from day 6 and continuing every second day thereafter (days 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, & 28)
- Daily body weight and survival

Treatment scheme

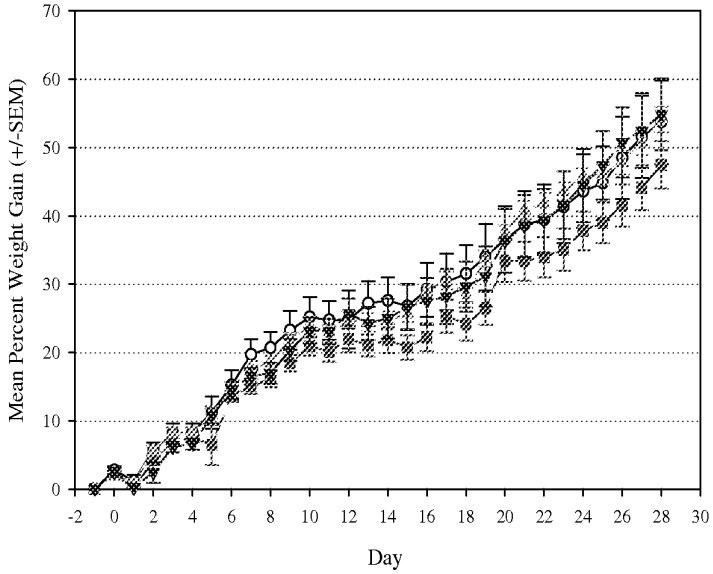
Group	N. of animals	Treatment	Route of Administration	Duration of Dosing
1	8/male	Untreated	NA	NA
2	8/male	Vehicle	IP	Days -1 to 15
3	8/male	Test Article 3 mg/kg	IP	Days -1 to 15
4	8/male	Test Article 30 mg/kg	IP	Days -1 to 15
5	8/male	Test Article 3 mg/kg	IP	Days -1 to 7
6	8/male	Test Article 30 mg/kg	IP	Days -1 to 7
7	8/male	Test Article 3 mg/kg	IP	Days 0 to 3
8	8/male	Test Article 30 mg/kg	IP	Days 0 to 3

Mucositis score table

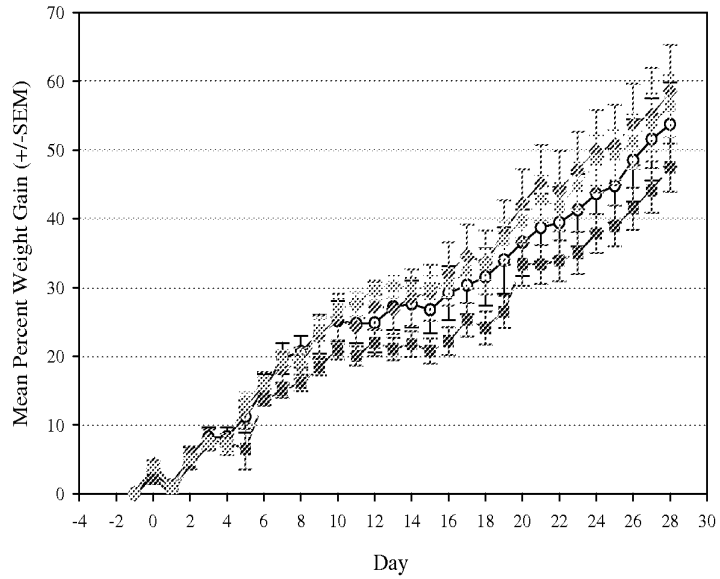
Score:	Description:
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray due to pseudomembrane. Cumulative size of ulcers should equal about ¼ of the pouch. Severe erythema and vasodilation.
4	Cumulative seize of ulcers should equal about ½ of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth)

Results: Body weight

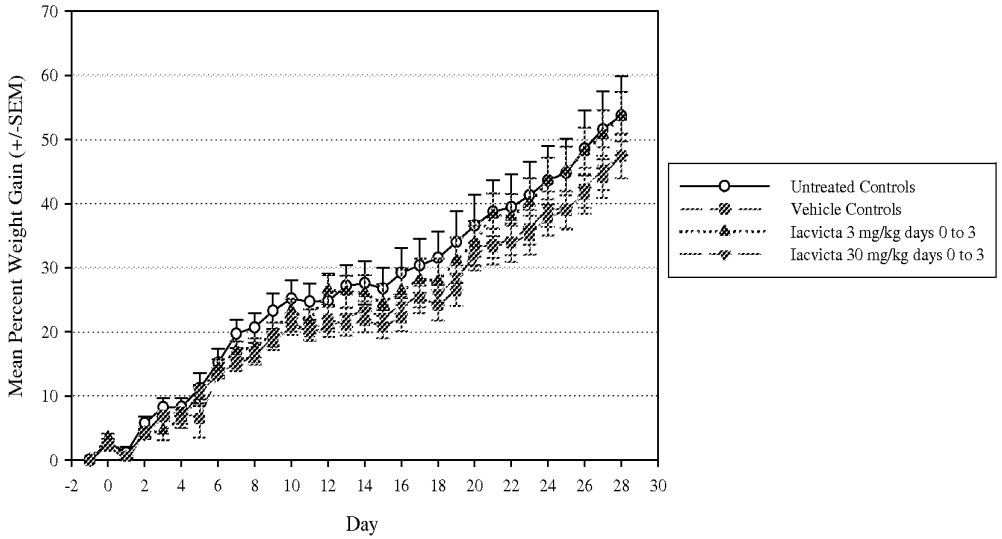
A



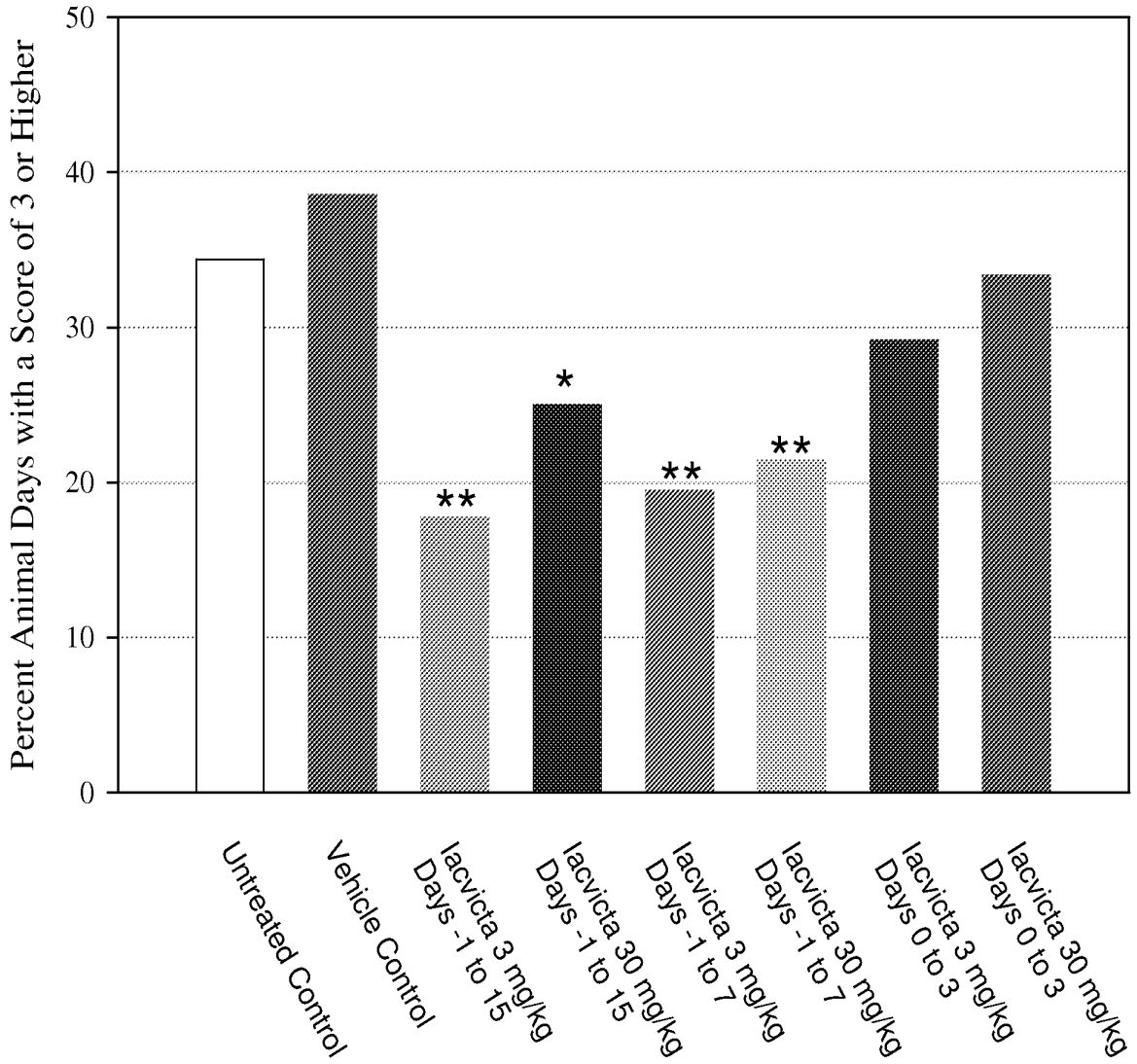
B



C



Results: Mucositis evaluation



The results are expressed as the percentage of days in which an animal exhibited an elevated score (greater than 3).

A single asterisk denotes statistically significant differences when compared to the vehicle. Dual asterisks denote statistically significant differences when compared to the both the untreated and vehicle controls groups.
$p < 0,05$ with Chi-squared tests.

Conclusions

- In the case lacvita was administered i.p. daily at the doses of 3 and 30 mg/kg before the induction of mucositis by irradiation (day -1), the mucositis score was statistically reduced when compared both to the vehicle and the untreated groups.

- lacvita treatment does not affect the body weight.

IAC gastro

EXHIBIT 9

eurofins

EUROFINS – Product Safety Laboratories

*The effect of **IAC** on an in-vivo Sepsis induction in rat model.*

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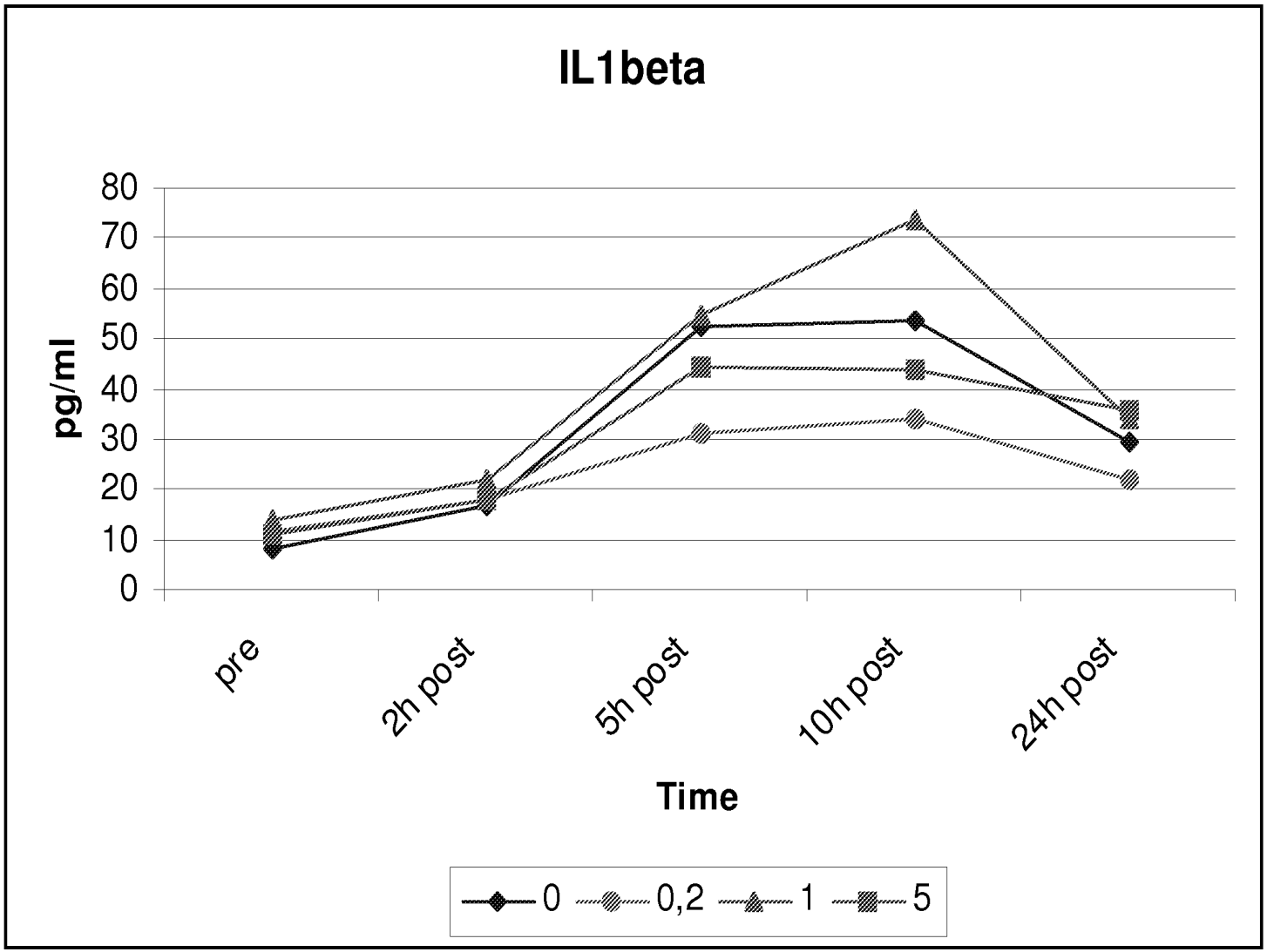
Study outline

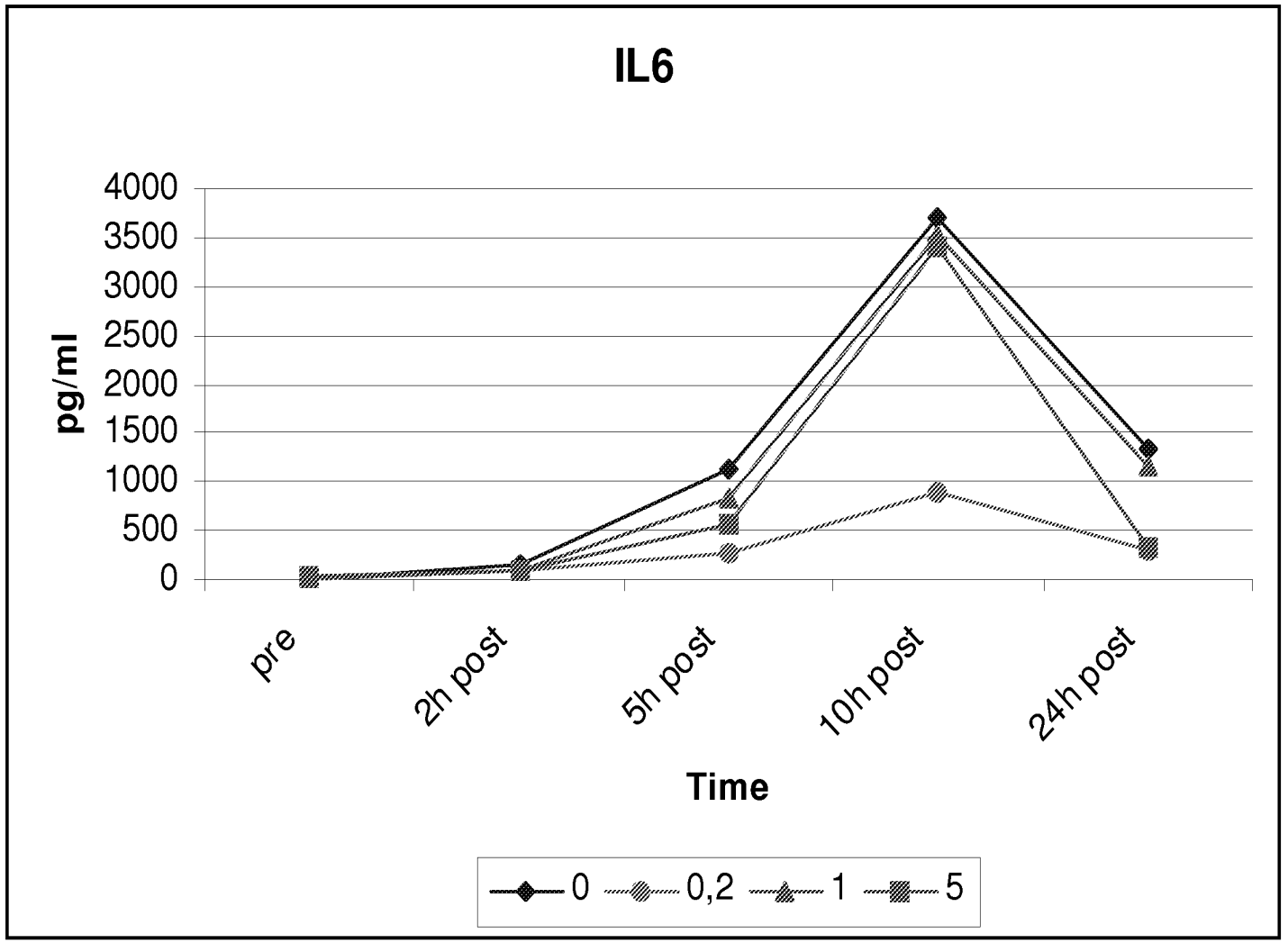
In vivo sepsis model in rat: cecal ligation and puncture model (CLP)

- 60 Sprauge-Dawley rats
- i.v. administration: day 0 post surgery, day 1, day 2 in tail vein
- 4 groups: vehicle, 0.2 mg/Kg, 1 mg/Kg, 5 mg/Kg
- Cytokines dosage: IL6, IL10, IL1beta
  - Pre-CLP, 2h post-CLP, 5h post-CLP, 10h post-CLP, 24h post-CLP
- Biochemistry dosage: LDH, AST, ALT, blood creatinine, urea nitrogen, albumin, K^+, Ca^{2+}, Na^+, Cl^-
  - 48h post-CLP
- Body weight
- Daily morbidity and mortality

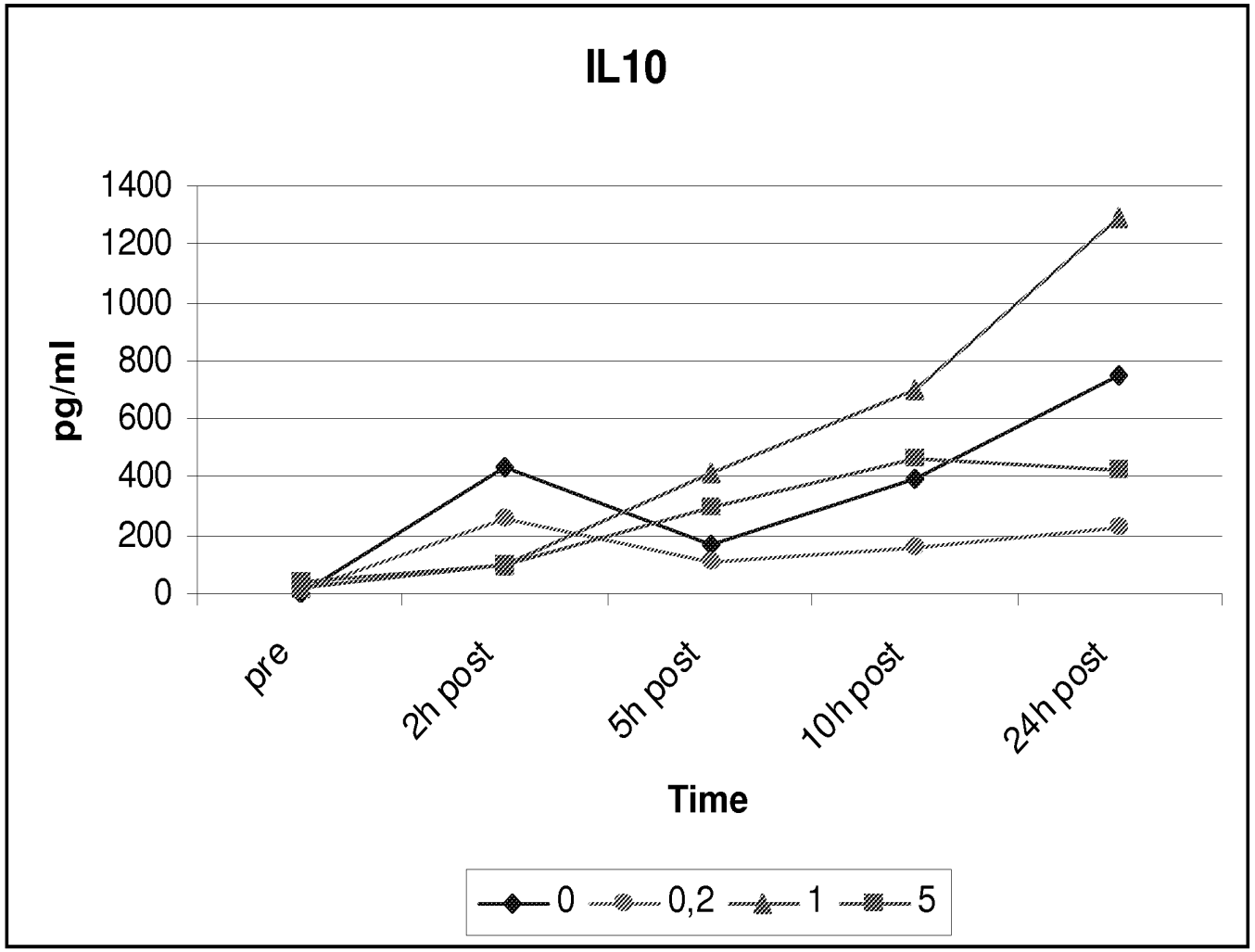
Results: Cytokines dosage



Results: Cytokines dosage



Results: Cytokines dosage



Results

Biochemistry dosage

IAC mg/Kg	ALT	AST	LDH	Albumin	BUN	Creatinine	Ca2+	Cl-	K+	Na+
0	217.8	511.6	483.3	2.3	63.8	0.8	9.7	94.3	5.2	133.8
0.2	139.7	289.3	399.8	2.4	37.7	0.4	10.0	92.2	5.8	132.4
1	225.2	434.8	480.3	2.2	36.3	0.4	9.5	96.5	5.8	136.0
5	246.2	653.4	648.0	2.2	95.6	0.9	9.8	88.8	6.1	131.2

Results

Mortality

	pre i.v.	post i.v. pre bleed	post bleed	pre i.v.	post i.v. pre bleed	post bleed		
IAC mg/Kg	o.n. D0 <24h	AM D1 24-28h	PM D1 28-36h	o.n. D1 36-48h	AM D2 48-52h	PM D2 52-60h	o.n. D2 60-72h	Lived
0	9	0	1	0	0	0	1	4
0.2	1	4	2	1	1	0	0	6
1	7	0	3	1	0	1	0	3
5	7	0	2	1	0	3	0	2

day0 day1 day2

IAC and vehicle administrations

Conclusions

- IAC significantly reduces mortality after one administration in the lowest treatment group.
- IAC shows a trend in reducing the cytokines IL6, IL1b levels
- IAC also may be induced a decrease in blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities.